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EFFECTS OF SPACE SHUTTLE EXHAUST PLUMES ON GILLS OF SOME ESTUARINE FISHES: A LIGHT AND ELECTRON MICROSCOPIC STUDY

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ABSTRACT The first few launches of the space shuttle resulted in fish kills in a lagoon near the launch site. To study this phenomenon further, sheepshead minnow (*Cyprinodon variegatus*), sailfin molly (*Poecilia latipinna*), and mosquitofish (*Gambusia affinis*) were exposed to the exhaust plume in buckets placed near the launch site. An open bucket provided a full exposure, a partly closed one provided an intermediate exposure, and a closed one was the control. Three h after launch, the pH of the water from the full exposure had decreased from about 7 to about 3, Al and Fe levels had increased, and some fish had died. Gills of most fishes from full exposures and some from intermediate exposures were damaged. Gills, however, exhibited no aneurysms, mucus coagulation, or hemorrhaging. Some secondary lamellae swelled, some fused with adjacent lamellae, and others clubbed or retracted into the filament. Many lamellar pavement cells died and sloughed off. Mucous cells of intermediate exposure specimens bulged on the filament surface and pavement cells lost their microridges. Mineral deposits, probably aluminum oxide, occurred on gills of fishes from full exposures. Focally, pavement cells were eroded exposing the underlying structures. The sudden pH drop in the full exposures probably caused the gill damage. However, we could not determine the effect of previous exposure on the experimental fish, or whether gill damage was the lethal lesion. The possibility is indicated that some fish recover after exposure to the exhaust plume.

INTRODUCTION

Kills involving fewer than one hundred small fish occurred in a lagoon following each of the first three launches of the Space Transportation System (STS; space shuttle) from Kennedy Space Center, Florida, USA. The cause of the fishes' deaths was not determined but was suspected to be gill damage resulting from a sudden drop in pH caused by the exhaust plume generated by the solid rocket boosters (Milligan and Hubbard 1983). Our preliminary histopathological examinations of whole fishes that were exposed to the exhaust plume of STS-4 in June 1982 confirmed those observations. We, therefore, designed an experiment to study further this unique interface between technology and estuarine ecology where an extreme stress condition develops and disappears rapidly. Our primary interest involved the effects of toxic agents or conditions on tissues, especially gills, of fishes exposed to the exhaust plume. In the present study, we examined whole fishes exposed to the space shuttle exhaust plume by light microscopy and gills by light microscopy, transmission electron microscopy, and scanning electron microscopy.

MATERIALS AND METHODS

Species studied included the mosquitofish *Gambusia affinis* (Baird and Girard); sailfin molly *Poecilia latipinna* (Leseur); Gulf killifish *Fundulus grandis* Baird and Girard; and sheepshead minnow *Cyprinodon variegatus* Lacépède. Two experiments were conducted in which fish were exposed to the exhaust plume: one during the launch of STS-4 in June 1982 and another during the launch of STS-5 in November 1982. To determine if previous exposure to the exhaust plume affected a fish's subsequent exposure, the species named above were collected from two sites, Molly Pond and an unnamed lagoon. Molly Pond (temperature 25°C, salinity 4 ppt), situated 6100 m and 260° west-southwest from the launch platform, was previously unaffected by shuttle launches. The lagoon (25°C, 14.5 ppt), a man-made body of water 400 m north from the platform, was the site of previous launch-related fish kills. For acclimatization, fish were transferred to two 75.7 liter aquaria with water temperature adjusted to 23–24°C and salinity to 10 ppt adjusted upward with Instant Ocean® and downward with deionized water. Artificial light hours occurred from 0800 to 1700 h daily. Fish were maintained 12–19 days before the experiment on a diet of TetraMin® flakes once each morning. Stressed fish were removed.

Fifteen and one-half h before the launch, fish were transferred to plastic buckets containing 10 liters of deionized water adjusted to 10–12 ppt. Five buckets contained 12 fish, one contained 10 fish, and each bucket included specimens of all three species. One set of buckets contained fish from Molly Pond and the other from the lagoon. In each set, one bucket was protected from the exhaust plume by a plastic cover (control exposure), one was open to the plume (full exposure), and one was covered with cheese cloth to provide a partial exposure (intermediate exposure). The buckets were placed 10–15 meters north of the pad perimeter fence, 3–4 meters west of the lagoon. This site is about 445 m north of the launch pad in a direct line with a concrete-lined flame trench that channels much of the exhaust plume away from the platform.

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TEXT-FIGURE 1

Water chemistry data from STS-5 fish experiment.

Exposure Specimen Source	pH	Temp (°C)	D.O.	Salinity (ppt)	AL (mg/l)	Fe (mg/l)
Control	–	–	–	–	<0.2	0.33
Closed						
Molly Pond	7.2	24.0	5.8	10.0	<0.2	0.22 ± 0.01
Lagoon	7.2	22.5	6.2	9.5	<0.2	0.2 ± 0.0
Intermediate						
Molly Pond	4.7	22.0	6.2	10.5	0.4 ± 0.0	0.58 ± 0.01
Lagoon	4.8	22.0	5.6	10.5	0.5 ± 0.0	0.75 ± 0.0
Open						
Molly Pond	2.9	21.0	6.0	10.0	0.8 ± 0.0	1.25 ± 0.07
Lagoon	2.8	22.0	6.0	11.0	1.3 ± 0.0	1.6 ± 0.0

Access to the experimental site was gained within 1.5 h after launch. Temperature, pH, conductivity, dissolved oxygen, and salinity of water were measured from each bucket. Water samples for heavy metal analysis by atomic absorption spectrophotometry were collected and placed on ice. Whole fish were fixed in 10% formalin. Specimens from which gills would be removed for ultrastructural examination were placed on ice to be dissected and fixed 5.5 h later. Gill arches, neither the first nor the last, were removed and fixed in ice-cold 3.0% phosphate-buffered glutaraldehyde for 3 h then placed in cold 0.1 M phosphate buffer and shipped on dry ice to Ocean Springs, Mississippi, for further processing and analysis which commenced 48 h later.

For transmission electron microscopy, gill arches were cut into small pieces, postfixed in 1% osmium tetroxide for 2 h and dehydrated in ethanol. After treatment with propylene oxide, the tissues were embedded in epoxy resin. For orientation and further light microscopical analysis, 1-micron thick sections were cut on an LKB ultramicrotome, mounted on glass slides, and stained with toluidine blue. Thin sections were cut on glass knives, collected on uncoated copper grids, stained with uranyl acetate and lead citrate, and examined in a Siemens 1A electron microscope.

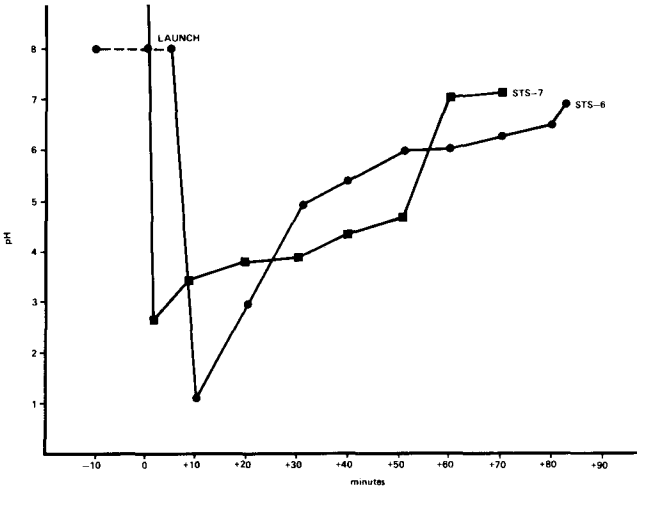
RESULTS

General Observations and Physical Parameters

The exhaust plume from each of the first five STS launches affected an area near to and north of the launch pad. A strong acidic odor, probably HCl, lingered and aluminum oxide powder covered much of the vegetation and together with dust blown from the launch gave the surface of the lagoon a tannish tint. Dead fish were usually found in dense grasses at the southern end of the lagoon in 15–30 cm of water.

Text-figure 1 shows water chemistry data from the six experimental buckets. Temperature, dissolved oxygen, and salinity changed little, if any, in the intermediate and full exposures. Aluminum and iron content increased about 2 to 4 times. There were no differences in the levels of other metals including Cd, Cr, Cu, Mn, Ni, Pb, and Zn. The pH, initially 7.2 in water from both the Molly Pond and the lagoon, decreased to 2.9 in the full exposure from Molly Pond and to 2.8 in the full exposure from the lagoon. Continuous pH measurements from two succeeding launches were recorded. The pH of the lagoon was between 8 and 9 before the launch and dropped to about 1 within 2–3 minutes following ignition (Text-figure 2). Within 60 minutes, the pH of the lagoon had recovered to about 7. In the Molly Pond full exposure, 2 of 12 specimens died, whereas 9 of 10 died in the lagoon full exposure.

Histopathological examination of paraffin-embedded whole fish revealed considerable damage involving gills but little, if any, involving olfactory, nervous, integumentary digestive, and hemopoietic tissues. Studies of peripheral



Text-figure 2. This graph represents continuous pH values taken during the launch of STS-7. The site was in the lagoon north of the launch pad. Water depth was 12 centimeters and pH was monitored at approximately 6 centimeters.

blood were not conducted, but blood in tissues other than gill did not appear affected. Since the gill appeared to be the major target organ, it was chosen for more extensive study.

Gill Morphology of Control Fish (Closed Bucket)

Gills of all three species appeared typical for teleosts at the organ, tissue, cell, and organelle level as seen by light microscopy (LM) and transmission electron microscopy (TEM) (e.g., Laurent and Dunel 1980), and in surface morphology as seen by scanning electron microscopy (SEM) (e.g., Hossler *et al.* 1979a). Most observations were made by LM of paraffin and epoxy-embedded specimens and by SEM. Sectioning difficulties caused by mineral deposits which occurred in interlamellar spaces of fish from intermediate and full exposures made TEM observations difficult to achieve. Observations could be made on broad areas of tissue but not on individual cells.

Gills of Molly Pond and lagoon fishes from control exposures showed no damage that might have indicated previous exposure to toxic conditions. Secondary lamellae were usually straight and interlamellar spaces free of deposits (Plate 1, Figures 1, 2). A narrow lymphatic space separated the smooth pavement epithelial layer from the underlying intralamellar capillaries. Red blood cells were normal. Short, irregular projections arose from lamellar pavement cells of some specimens. Filaments of control gills contained undifferentiated cells, mucous cells, and chloride cells and often exhibited large, apparently intercellular, spaces. Mucous cells were especially numerous on the filament surface associated with the efferent artery whereas chloride cells were abundant near the filament surface associated with the afferent artery and in interlamellar regions (Plate 1, Figure 3). Chloride cells stained lightly and had basally-situated nuclei. The identity of chloride cells was confirmed by TEM. Most chloride cells appeared to have deep apical pits opening to the filament surface.

Gill lamellae of control specimens were thin, regular, overlapping plates with relatively smooth surfaces without pores or microridges (Plate 1, Figure 4). The filament surface, however, had numerous pores, the majority of which probably represent apical pits of chloride cells. Microridges were usually absent from the center of pavement cell surfaces, but abundant near the peripheries (Plate 1, Figure 5).

Changes in Gills of Fishes from Intermediate Exposures

Secondary lamellae showed no disruption or erosion, but some were angulated (Plate 2, Figure 6) and swollen. None of the fish died and no conspicuous differences in the types of pathological changes occurred among species. Some lamellae appeared thickened and retracted into the filament (Plate 2, Figure 7). Secretory granules of some mucous cells on interlamellar regions of filaments were denser than in others. Mucous cells moved to the surface and appeared to bulge outward (Plate 2, Figure 8). Interlamellar spaces of some fish had mineral deposits that ranged from granules less than 1.0 μm in diameter to aggregates more than 40.0 μm in diameter. Pathological changes in gills, however, did not relate to the presence of granules.

Numerous broad, shallow depressions and some deeper ones occurred near the interlamellar region (Plate 2, Figure 9). Mucous cell surfaces were usually smooth but sometimes had small granules (about 0.5 mm in diameter) attached (Plate 2, Figure 10). Such granules occurred often on pavement epithelial cells, especially microridged portions. Little change occurred in the microridge patterns of the pavement epithelial cells.

Changes in Gills and Other Organs of Fish from Full Exposures

Histopathological changes varied among specimens, arches, and filaments, often involved small portions of a few lamellae, and ranged from apparently mild to potentially lethal ones.

Histopathological changes exhibited in some dead specimens that had not autolyzed appear relevant. In a few paraffin-embedded specimens that exhibited severe gill necrosis and had died following the exposure, blood spaces of the heart, particularly the atrium, were congested and the pericardial cavity filled with a transudate (Plate 3, Figure 11).

Only one of four fish, a sailfin molly, from full exposures examined in epoxy-resin sections had not died following the exposure. In this fish, secondary lamellae shortened and epithelial cells lifted away from underlying tissues creating broad lymphatic spaces (Plate 3, Figure 12). Mineral deposits occurred in many interlamellar spaces. Apices of mucous cells lay near the surface of the nonlamellar portion of the filament, and many mucous granules stained less densely with toluidine blue than did those from control or intermediate exposures (Plate 3, Figure 13). Changes in gills of fish that died in full exposures included fusion of adjacent lamellae, clubbing of the ends of lamellae, hemostasis in afferent and efferent filament vessels and in lamellar capillaries, and erosion of cells of secondary lamellae (Plate 3, Figure 14). TEM confirmed that the eroded cells were pavement epithelial cells (Plate 3, Figure 15).

Focal lesions involved primarily lamellae in the distal two-thirds of filaments (Plate 4, Figure 16). In some places, the surface epithelium eroded away exposing the underlying filament vessels and lamellar capillaries (Plate 4, Figure 17). Mineral deposits occurred frequently. Neither microridges nor pores were often seen on filaments of these fish (Plate 4, Figure 18).

Fish that died in full exposures exhibited many of the changes seen in fish that had not. This included erosion of epithelial layers. Lamellae in these fish frequently fused. Plate 4, Figure 19 shows a small area of lamellae exhibiting several degrees of fusion. In some places, fusion occurred between broad areas of epithelium and, in other places, among individual cells. Numerous bulges in the lamellae probably represent nuclei of pavement epithelial cells.

DISCUSSION

Few field studies have been conducted to determine the ultrastructural effects of toxicants on fishes. Optimally, specimens for such studies are collected, fixed, and processed rapidly. Hughes et al. (1978, 1979) developed morphometric techniques for determining subtle effects of pollutants on fish gills and emphasized the importance of consistent and appropriate fixation and processing protocols for specimens used for morphometry. In our study, logistical problems including delayed access to the experimental site, a delay in fixation (although specimens were kept on ice), and a delay in processing caused by having to ship the tissues to a second laboratory were unavoidable. Such factors could obviate morphometrical analyses but not qualitative ones, providing adequate control specimens are examined. Even examination of dead specimens can give useful information.

All experimental fish exposed to the exhaust plume had severely damaged gills. Damage consisted mainly of necrosis and sloughing of pavement cells of secondary lamellae. Other histological changes included swelling and clubbing of secondary lamellae, loss of microridges from the filament pavement cells, and mucus secretion. These changes were probably caused by sudden exposure to acid conditions as recorded in buckets exposed to the exhaust plume. Additional measurements taken during the launches of STS-6 and STS-7 confirmed this pH decline in the lagoon near the experimental site. The ignition of the two solid rocket boosters and the simultaneous release of several thousand kiloliters of deluge water result in the formation of gases and particulates including carbon dioxide, aluminum oxide, water vapor, hydrogen chloride, and iron chloride. Hydrogen chloride gas mixes with the ambient air and is readily scavenged by atomized water droplets and small drops which form from condensation as the exhaust plume cools. Most of the larger drops, possessing a pH of less than 0.5, are deposited near the pad (Keller and Anderson 1983). Our study indicates that exhaust plume components exert their primary histopathologic effects on gills of exposed fishes. This confirms an earlier preliminary study by Milligan and Hubbard (1983).

Accurate diagnosis of gill effects must be based on examination of large numbers of filaments because effects vary widely in different parts of a gill arch (Fromm 1980). SEM can help overcome some of these sampling problems and, when used in combination with LM and TEM, rather fine changes can be determined in specific cell types such as pavement epithelial cells, chloride cells and mucous cells.

Some subcellular aspects of the histopathological response of gills to toxic conditions deserve comment. Filament pavement cells of *G. affinis, C. variegatus,* and *P. latipinna* had microridges, but those on secondary lamellae did not. Similarly, Hossler et al. (1979a) reported that secondary lamellae of mullet *Mugil cephalus* Linnaeus lacked microridges. Microridges, however, were reported on secondary lamellae of the catfish *Heteropneutes fossilis* (Bloch) by Rajbanshi (1977), and the dogfish *Scyliorhinus canicula* Linnaeus by Crespo (1982). The case of the rainbow trout *Salmo gairdneri* Richardson is not clear. Kendall and Dale (1979) reported no microridges on secondary lamellae, whereas Olson and Fromm (1973) and Hughes (1979) reported them to be present. More species under different exposure regimes and fixation procedures need to be examined by SEM to determine the nature and possibly the functions of these structures. In heat-stressed rainbow trout, the loss of microridges on gill surfaces was attributed to increased mucus production filling the depressions between the microridges (Jacobs et al. 1981). Jagoe and Haines (1983) reported that microridges on gills of Sunapee trout *Salvelinus alpinus oquassa* dissappeared after exposure to pH 3 for 4 hours. They suggested that cellular swelling or membrane alterations were responsible. Cellular swelling generally occurs after acutely injured cells lose cell volume regulation (Trump and Ginn 1969). We consider the loss of microridges in fish exposed to space shuttle exhaust plume to be part of a spectrum of changes that probably eventually leads to necrosis. LM, TEM, and SEM did not reveal an excess of mucus among microridges of the fish we examined.

Fromm (1980) reviewed the effects of acid stress on freshwater fish and concluded that death may be caused by hypoxia brought on by alteration of gill membranes, coagulation of gill mucus, or a combination of the two. Gill mucus coagulation did not occur in the present study. However, mucus was apparently discharged in exposed fish. Daye and Garside (1976) found that stress by pH caused hypertrophy and stimulated mucus secretion in gills of brook trout *S. fontinalis.* We did not see hypertrophy of mucous cells, but the heterogeneity of secretory granules in intermediate and full exposure groups suggested to us that those cells had been stimulated to release their stored mucus granules and had begun replacing them.

Several hypotheses have been advanced to explain the role of mucus secretion as a protective response to acid stress. The benefit of mucus to the stressed fish might depend on the mucus being a barrier to ions and water, its being polyanionic and concentrating cations, or its specific binding of calcium, which is important for maintenance of permeability control (see review by McDonald 1983).

Damage to secondary lamellae must be considered potentially serious because of the possible effects on respiration. Hughes and Morgan (1973) reviewed the general histopathological responses of secondary lamellae to pollutants. An initial response is thickening of the gill epithelium due to swelling in acute exposures or to cell proliferation in long term exposures. Next, secondary lamellae fuse, the pavement cell layer lifts, and pavement cells dissociate. Epithelial lifting might help protect the gill from a toxicant

by increasing the diffusion distance between the ambient water and the fish's blood (Morgan and Tovell 1973) or by limiting water circulation between gill lamellae. In the present study, fusion of lamellae occurred not only between broad sheets of epithelium, but also processes of individual epithelial cells bridged the interlamellar space and joined adjacent lamellae. Using SEM, Engelhardt et al. (1981) reported fusion between broad areas of lamellae in rainbow trout exposed to crude oil emulsions whereas Jacobs et al. (1981) illustrated a focal type of fusion between secondary lamellae of heat-stressed rainbow trout. Possibly, the most severe gill injury that we saw in full exposures consisted of sloughing of epithelial cells of secondary lamellae, sometimes exposing the underlying capillary network. A similar effect was noted by Daye and Garside (1976) in secondary lamellae of rainbow trout exposed to environments at and above pH 9.0 and below 5.6.

Chloride cells, which are involved in monovalent ion regulation, occur in both euryhaline and stenohaline species of freshwater and marine fishes. These cells are located mainly in the interlamellar regions of filaments but in marine and seawater-adapted species also along the surface of the filament related to the afferent filament artery (Laurent and Dunel 1980). Hossler et al. (1979b) showed with SEM that the numerous pores on filament surfaces of seawater-adapted *M. cephalus* represented apical pits of chloride cells. In freshwater-adapted specimens, pores were shallower, and cytoplasm of chloride cells extended above the level of the pavement epithelium. We confirmed the identity of chloride cells by LM and TEM. With SEM, changes in the size, distribution or depths of filament pores after exposure could not be documented. Some filament pores, however, might represent evacuated mucous cells.

Tissue damage does not necessarily indicate the cause of a fish's death (Hughes and Morgan 1973). In heat-killed specimens of the banded killifish *Fundulus diaphanus* (Lesueur), Rombough and Garside (1977) considered the cause of death to be respiratory failure resulting from lesions in the medulla oblongata despite the presence of primary gill injury. For acutely lethal concentrations of some toxicants, however, death might not be accompanied by tissue damage. In gills of rainbow trout exposed for 2.5 h to lethal concentrations of ammonia, Smart (1976) reported neither increased mucus production nor hemorrhage. He concluded that gill damage was not the cause of death in that situation.

Whether fish surviving the acute exposure to the exhaust plume can recover is not known with certainty. The time course and mechanisms involved in repair of damaged tissues must be determined in controlled, laboratory experiments. Lloyd and Jordan (1964) found that rainbow trout that survived exposure to pH 3.8 for 24 h recovered when transferred to clean water. Recovery from the exhaust plume, however, is indicated by several factors. First, fish kills that are caused by the exhaust plume occur abruptly, and fish do not continue to die afterwards. Second, examination of fishes from the lagoon area that had been the site of previous kills revealed no latent pathologic changes that might have been related to previous exposures to the exhaust plume. However, lagoon fish might have been more susceptible to subsequent exhaust plume exposure since a much higher percentage of them died in full exposures than did fish from Molly Pond.

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PLATE 1

EXPLANATION OF FIGURES

1. ***Cyprinodon variegatus*** **from control exposure. Filament (F); secondary lamellae (L). Paraffin section; hematoxylin and eosin stained. X 430.**
2. ***C. varigatus*** **from control exposure. Chloride cell (C); red blood cells (R). Epoxy resin section; toluidine blue stained. X 430.**
3. ***C. variegatus*** **from control exposure. Note chloride cell with central opening (*) probably representing apical pit. Mucous cells (M). Epoxy resin section; toluidine blue stained. X 430.**
4. ***C. variegatus*** **from control exposure. Secondary lamellae (L); filament (F). Scanning electron micrograph. X 370.**
5. ***C. variegatus*** **from control exposure. Pores (P) on filament surface are probably those of chloride cells. Note microridge patterns (arrowheads) that delineate individual pavement epithelial cells. Scanning electron micrograph. X 3,660.**

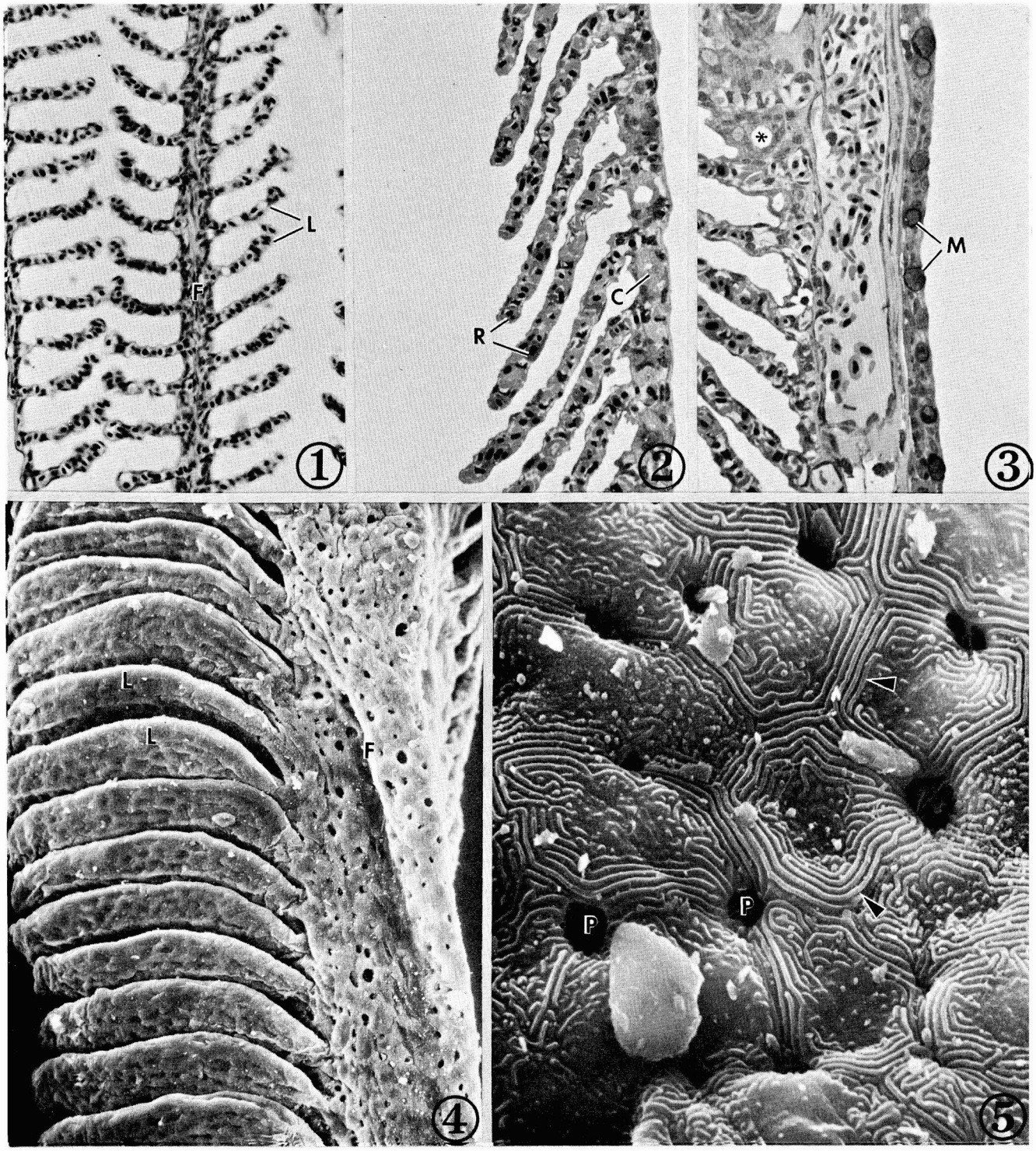

PLATE 2

EXPLANATION OF FIGURES

6. *Cyprinodon variegatus* from intermediate exposure. Note angulation of secondary lamellae and that the lamellae are somewhat thicker than control lamellae in Figures 1–3. Epoxy resin section; toluidine blue stained. X 430.

7. *C. variegatus* from intermediate exposure. Note shortening, wrinkling, and swelling of secondary lamellae. Also, mucous cells (M) have different densities. Filament cartilage (FC). Epoxy resin section; toluidine blue stained. X 430.

8. *C. variegatus* from intermediate exposure. Note mucous cells (arrowheads) on filament surface bulge outward slightly. Epoxy resin section; toluidine blue stained. X 430.

9. *C. variegatus* from intermediate exposure. Note depressions (*) in interlamellar regions of filament. Scanning electron micrograph. X 730.

10. Enlargement of filament surface shown in Figure 9. Note numerous bulging cells, probably mucous cells (M) and many pavement epithelial cells partially devoid of microridges (*). Chloride cell pore (P). X 1,830.

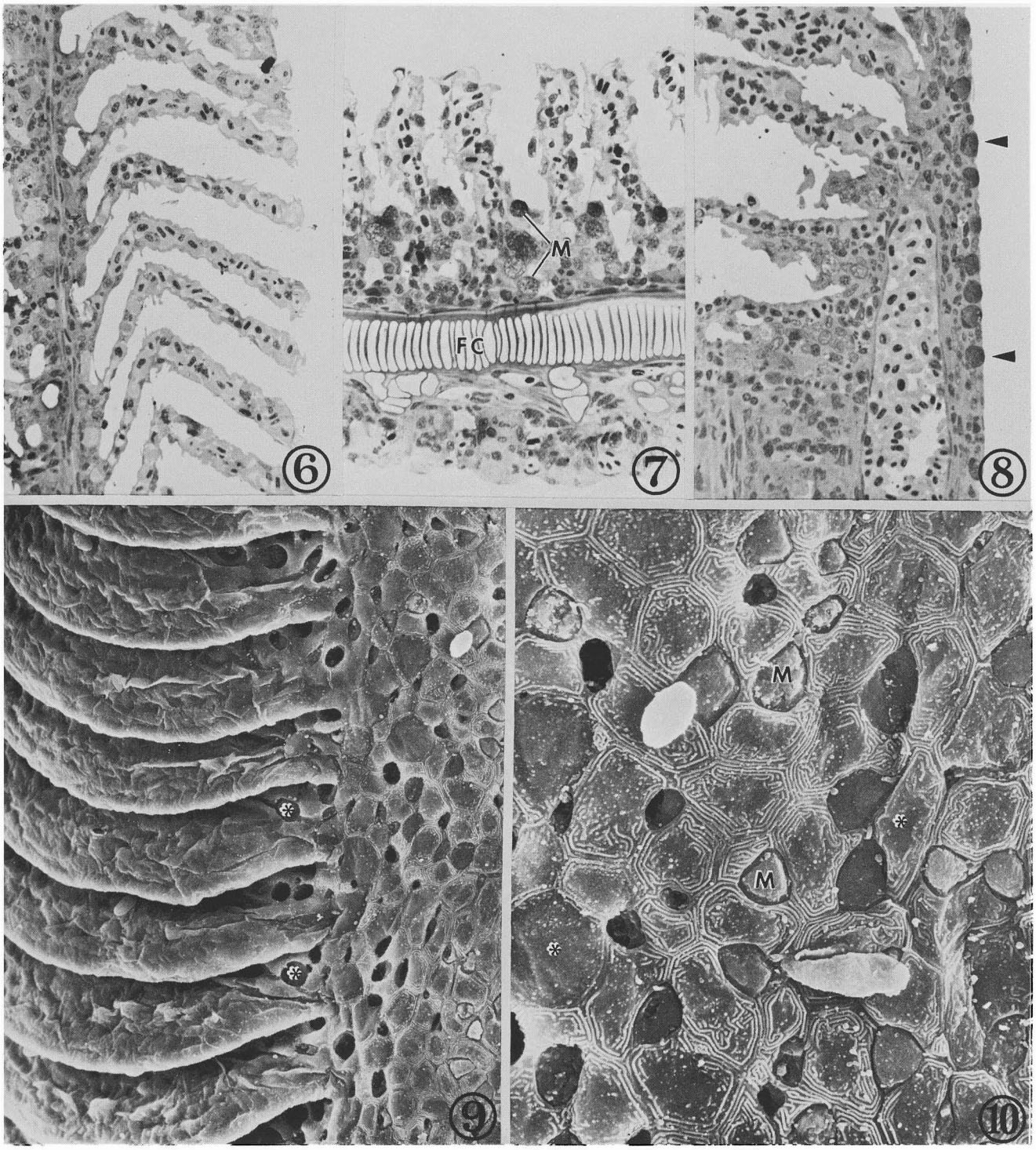

PLATE 3

EXPLANATION OF FIGURES

11. *Poecilia latipinna* that died from full exposure. Note blood congestion in atrium (A) of heart and transudate in pericardial space (PS). Paraffin section; hematoxylin and eosin stained. X 70.

12. *P. latipinna* from full exposure. Note retracted lamellae, wrinkling of pavement epithelial cells (arrowheads) and areas of epithelial lifting (*). Epoxy resin section; toluidine blue stained. X 430.

13. *P. latipinna* from full exposure. Note mucous cells (M) are lightly staining. Epoxy resin section; toluidine blue stained. X 430.

14. *Cyprinodon variegatus* that died from full exposure. Lamellae have fused so that normal architecture is disrupted. Epoxy resin section; toluidine blue stained. X 430.

15. *C. variegatus* that died from full exposure. Pavement epithelial cells (PC) are necrotic and separated from secondary lamella. Transmission electron micrograph. X 10,380.

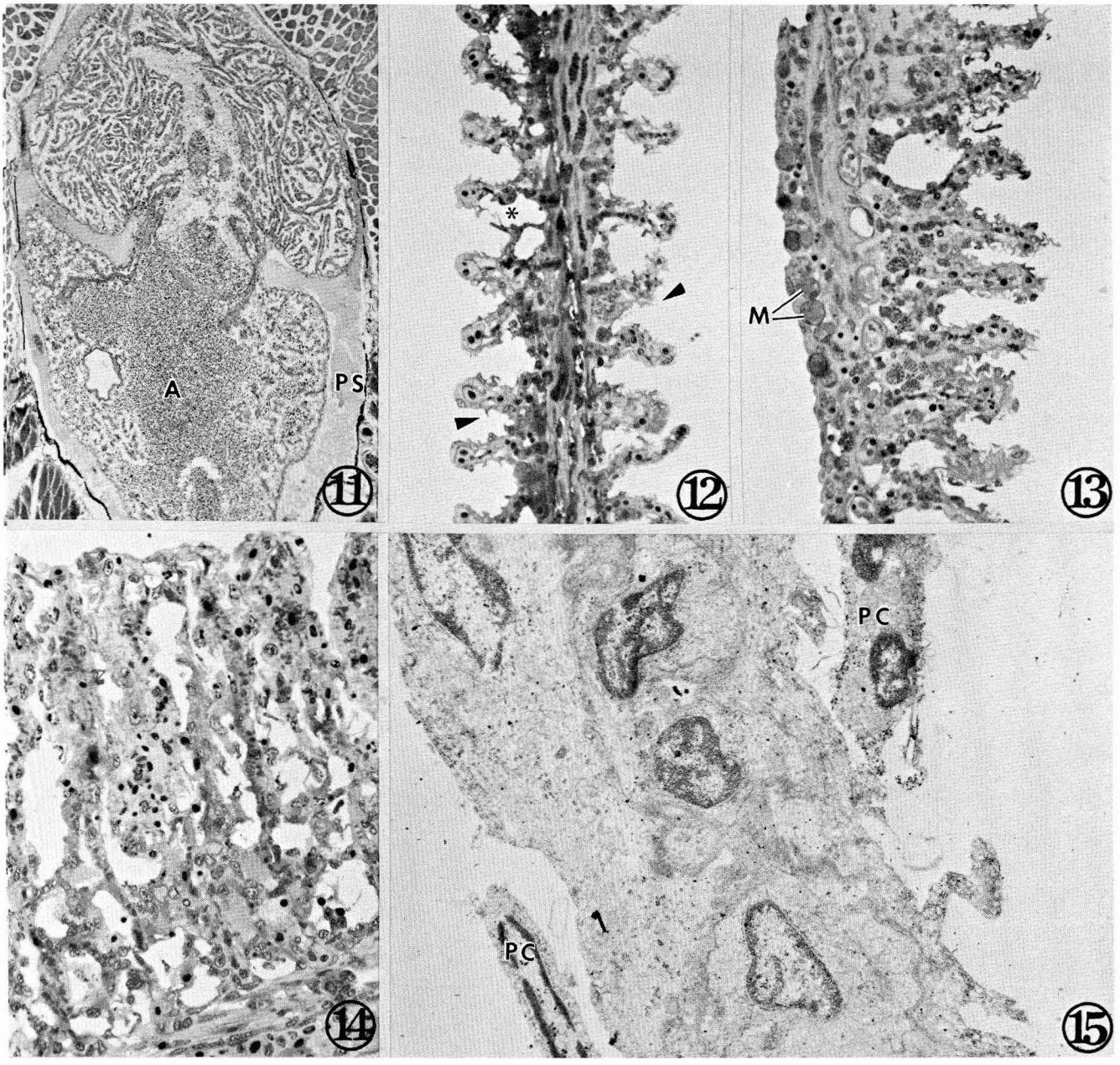

PLATE 4

EXPLANATION OF FIGURES

16. *Poecilia latipinna* from full exposure. Note areas of damaged secondary lamellae (arrow). Gill arch (A); mineral deposits (MD). Scanning electron micrograph. X 210.

17. *P. latipinna* from full exposure. Loss of superficial epithelium reveals artery (FA) and lamellar capillaries (LC). Scanning electron micrograph. X 830.

18. *P. latipinna* from full exposure. Note absence of microridges from filament surface (F). Scanning electron micrograph. X 830.

19. *C. variegatus* that died from full exposure. Note fusion between broad areas of lamellae (*) and between narrow cellular bridges (arrows). Nuclei of pavement epithelial cells (arrowheads). Mineral deposits (MD). X 830.