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# STUDIES ON THE STRUCTURE AND FUNCTION OF THE TELEOST PSEUDOBRANCH

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Studies on the Structure and Function of the Teleost Pseudobranch

by

Derek L. Mattey

### VOLUME II



Experimental set-up for pseudobranch nerve preparation

1-1 m, manometer; o, oscilloscope; r, reservoir
1-2 c, catheter tubing; ch, cooling chamber; e, electrode; mm, micromanipulator; t, three way tap.

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Position of the pseudobranch in <u>Salmo gairdneri</u> (Rainbow trout)

- 2-1 Left pseudobranch (ps) supported by the internal opercular surface (o); g, first gill arch; n, pseudobranch nerve just visible beneath the epithelium.
- 2-2 Gill arches removed after microfil injection to show the blood supply of the pseudobranchs (ps); apa, afferent pseudobranchial artery; epa, efferent pseudobranchial artery (x 7.3).
- 2-3 Light micrograph of the pseudobranch (ps) 'in-situ' showing its relationship to the gill arches (g). The pseudobranch is situated on a base of adipose tissue (ad) covering a layer of muscle (m). epa, efferent pseudobranchial artery; ep, epithelial covering (Mallory's triple stain x 34).



Light micrographs of representatives of the 4 main types of pseudobranch (Mallory's triple stain).

3-1	Free pseudobranch of <u>Dincentrarchus</u> <u>labrax</u> (Bass) (x 128)
3 <b>-</b> 2	Semi-free pseudobranch of <u>Mugil capito</u> (Grey mullet) (x 112)
3 <b>-</b> 3	Covered pseudobranch of <u>Salmo gairdneri</u> (Rainbow trout) (x 85)
3-4	Buried pseudobranch of <u>Cyprinus carpio</u> (Common carp) (x 102) ad, adipose tissue; afa, afferent filament artery; ca, cartilage; cc, position of 'chloride type' cells; efa, efferent filament artery; m, mucous cells; pc, position of 'pseudobranch type' cells; sl, secondary lamellae; vc, venous channel.



Surface epithelial cells.

- 12-1 Surface of pseudobranch arch (bass). The surface of the epithelial cells (e) consists of numerous microridges, arranged in concentric whorls (x 1,446).
- 12-2 Surface of mullet pseudobranch filament. The epithelial cells may possess microridges (mr) or microvilli (mv), and are separated by long straight ridges (r). Mucous pores (mp) are seen at the junction of epithelial cells (x 3,342).



- 13-1 T.S. of bass pseudobranch. afa, afferent filament artery; ala, afferent lamellar arteriole; bc, blood channels of secondary lamella (sl); ca, cartilage; cvs, central venous sinus; efa, efferent filament artery; p, pillar cells (Methylene blue x 615).
- 13-2 Afferent filament artery of bass pseudobranch. ca, cartilage; bl, basal lamina; en, endothelial cells; m, muscle cell (x 3,750).
- 13-3 Efferent filament artery of bass pseudobranch. co, collagen fibres; en, endothelial cells; m, muscle cell; rbc, red blood cell (x 3,990).



Comparison of gill filaments and pseudobranch filaments in the bass.

- 4-1 Light micrograph of gill filaments (f). Note the cartilaginous rod (ca), gill rakers (gr) and secondary lamellae (sl) (Mallory's x 59).
- 4-2 Light micrograph of pseudobranch filaments (f). Note the cartilaginous rod and thick secondary lamellae (sl) Mallory's x 122).



Scanning electron micrographs to show the buccal and opercular edges of the bass pseudobranch filament.

- 5-1 Buccal edge (x 624).
- 5-2 Opercular edge (x 510). e, epithelial cells; f, filament; fs, area of epithelial fusion covering secondary lamellae; m, mucous cell pores; sl, secondary lamellae; t, filament tip.



Mullet pseudobranch filament.

- 6-1 Light micrograph of filament showing position of 'chloride type' cells (cc), 'pseudobranch type' cells (pc), secondary lamellae (sl), epithelial covering (ep) and filament tip (t) (Haematoxylin & eosin x 196).
- 6-2 Scanning electron micrograph of filament tip showing serrated appearance of secondary lamellae (sl) on the underside of the filament (opercular edge) cp, 'chloride' cell pit; ep, epithelial cells; t, filament tip (x 1,125).



Mullet pseudobranch.

- 7-1 Mullet pseudobranch filament (buccal edge).
  d, dome-like protrusions; e, epithelial cells;
  m, mucous pores (x 687).
- 7-2 Light micrograph of mullet pseudobranch filament (TS) showing position of 'chloride type' cells (cc) and 'pseudobranch type' cells (pc). ca, cartilage; efa, efferent filament artery; ep, epithelial cells; sl, secondary lamellae (free region) (Methylene blue x 372).



Light micrographs of the covered pseudobranch of rainbow trout.

- 8-1 The secondary lamellae (sl) are fused along the length of the filaments (Haematoxylin and eosin x 99).
- 8-2 An epithelial layer (ep) covers the filament. b, blood channel; m, mucous cell; sl, secondary lamellae (Methylene blue x 371).
- 8-3 The filament is not attached to the operculum at its tip. ca, cartilage; ep, epithelial covering; m, mucous cells; sl, secondary lamellae (Mallory's x 169).



Light micrographs of buried pseudobranchs.

- 9-1 Pseudobranch of <u>Pollachius pollachius</u> (Pollack). Note folded, lobulated appearance of the filaments. ad, adipose tissue; m, muscle; pea, primary efferent artery; sl, secondary lamellae (Mallory's x 52).
- 9-2 Pseudobranch of <u>Cyprinus carpio</u> (Carp). ad, adipose tissue; efa, efferent filament artery; pea, primary efferent artery; sl, secondary lamellae; (Mallory's x 80).



Light micrograph of the pseudobranch arch of bass (Mallory's x 242).

ad, adipose tissue; c, connective tissue; ca, cartilage; m, mucous cells; paa, primary afferent artery; pea, primary efferent artery; pv, pseudobranchial vein; sl, secondary lamellae.



- 11-1 Large and small nerve bundles, and capillaries run between the fat cells of the pseudobranch connective tissue. ad, adipose tissue; n, nerve bundle (Haematoxylin & eosin x 206).
- 11-2 Taste bud (tb) in the opercular epithelium of rainbow trout. ep, epithelium (H & E x 448).
- 11-3 Loose connective tissue of the carp pseudobranch.
   f, cytoplasmic tibres; fb, fibroblast; g, granule
   of mast cell (mc); nu, nucleus; s, tissue space;
   st, strand of fibroblast (x 8,934).
- ll-4 Lipid droplet (ld) in trout pseudobranch adipose tissue surrounded by nerve bundles (n) (x 6,144).



14-1 Channels of the central venous sinus in the trout pseudobranch. c, connective tissue; co, collagen fibres; en, endothelial cell; vc, venous channel (x 4,884).

14-2 Lining of the central venous sinus (cvs) in trout pseudobranch. bm, basement membrane; co, collagen fibres; en, endothelial cells (x 4,725).

14-3 Region of the central venous sinus close to the adventitia of the afferent filament artery. A small nerve bundle (n) is associated with an arteriole (ar). 'Secretory type' cells containing electron dense granules (g) are also seen. vc, venous channel (x 19,500).



- 15-1 Filament epithelium of bass pseudobranch consisting of layers of unspecialised cells, and a mucous cell (m), with dense droplets, near the epithelial surface, mr, microridges (x 5720).
- 15-2 Surface of the filament epithelium of bass pseudobranch. d, droplets of mucous; mp, mucous pores; mr, microridges (x 6,957).



- 16-1 Surface epithelial cells of bass pseudobranch filament. d, desmosomes; dg, dense granules; go, Golgi apparatus; j, junction between two epithelial cells; mr, microridges; sep, surface epithelial cell (x 11,605).
- 16-2 Surface epithelial cells of bass pseudobranch secondary
  lamella.
  d, desmosomes; g, glycocalyx; mr, microridges (x 14,364).
- 16-3 Glycocalyx (g) of surface epithelial cells stained with silver methenamine (x 36,400).



- 17-1 Endothelial cell (en) of an afferent lamellar arteriole in the bass pseudobranch. go, Golgi complex; gr, dense endothelial granule; vb, multivesicular body (x 30,640).
- 17-2 Junction between two endothelial cells (en).
  b, basal lamina; d, desmosomes; f, bundle of filaments;
  gr, specific endothelial granule (x 35,750).
- 17-3 Foot-like process (fo) emerging from the base of an endothelial cell (en). m, muscle cell; c, collagen fibres (x 35,750).


18-1 Junction of the afferent filament artery (afa) and afferent lamellar arteriole (ala) in bass pseudobranch. b, basement membrane; ca, cartilage; gr, specific endothelial granules of endothelial cells (en); m, muscle cells (x 4,500).

18-2 Endothelial cells (en) at the junction of an afferent filament artery and afferent lamellar arteriole in bass pseudobranch. co, collagen fibres; go, Golgi complex; gr, specific endothelial granules; n, nerve bundle (x 7,125).

18-3 Pillar cell (p) in the secondary lamella of trout pseudobranch. bm, basement membrane; c, column; co, collagen (x 19,500).



- 19-1 Pillar cell in the secondary lamella of bass pseudobranch. c, column; f, cytoplasmic fibrils; nu, nucleus; m,mitochondria; v, vesicles (x 7,050).
- 19-2 Marginal channel (mc) in a secondary lamella of the bass pseudobranch. en, endothelial cell; gr, specific endothelial granule; p, pillar cell; pc,' pseudobranch type'cell (x 7,100).



Epithelial cells covering the secondary lamellae of the bass.

- 20-1 Epithelial cells at the marginal base of the secondary lamellae, close to the filament. mr, microridges; mv, microvilli; p, possible 'chloride' cell pit (x 6,435).
- 20-2 Epithelial cells covering the 'pseudobranch' cells of the bass pseudobranch. mr, microridges (x 3,986).



- 21.1 Chloride cell pits (cp) of bass pseudobranch secondary lamellae. mr, Microridges (x 3,664).
- 21.2 'Free' area of mullet pseudobranch secondary lamellae showing chloride cell pits (cp), microridges (mr) and microvilli (mv) (x 2,504).



Interstitial cells of the trout pseudobranch secondary lamellae.

22-1 Interstitial cell in close contact with a nerve bundle (n). The cell contains extensive bundles of fibres (f).

d, desmosome; nu, nucleus (x 29,120).

- 22-2 Interstitial cell between 'pseudobranch type' cells (pc). m, mitochondria; nu, nucleus (x 12,000).
- 22-3 Interstitial cell containing a thick bundle of fibres (bf) and making contact with a 'pseudobranch type' cell by means of desmosomes (d) (x 36,400).



- 23-1 Light micrograph of bass pseudobranch filament (T.S.). afa, afferent filament artery; bc, blood channel, ca, cartilage; cc, chloride cells; cco, central compartment; sl, secondary lamellae (Methylene blue x 512).
- 23-2 'Chloride type' cells (cc) in the secondary lamellae of bass pseudobranch. ap, apical pit; bc, blood channel; bm, basement membrane; ep, flattened epithelial cells; ger, granular endoplasmic reticulum; m, mitochondria; mu, mucous cell; s, saccules (x 4,749).



- 24-1 Secondary lamellae (sl) on the gill filament of bass. bc, blood channel; cc, 'chloride' cell; m, mucous cell; r, rodlet cell; vc, venous channel (Methylene blue x 1,010).
- 24-2 Base of secondary lamella on the gill filament of bass. ap, apical pit; bc, blood channel; bm, basement membrane; cc, 'chloride' cell; ep, epithelial cell (x 3,315).



- 25-1 'Chloride type' cell of bass pseudobranch. ap, apical pit; ac, accessory cell; bm, basement membrane; ger, granular endoplasmic reticulum; m, mitochondria; n, nucleus; s, saccules; t, tubules (x 11,147).
- 25-2 Base of 'chloride type' cell of bass pseudobranch, bc, blood channel; bm, basement membrane; s, saccules; t, tubules (x 41,924).



- 26-1 Lattice-like arrangement (1) of tubules (t) connected to narrow saccules (s) at the base of a pseudobranch 'chloride type' cell (x 59,400).
- 26-2 Apical region of bass 'chloride type' cell. ac, accessory cell; cp, clear cytoplasmic process; ep, epithelial cell; j, shallow junction between accessory cell and 'chloride type' cell; t, tubules; v, clear vesicle (x 17,094)
- 26-3 Dark 'chloride type' cell containing an extensive granular endoplasmic reticulum (ger). ac, accessory cell (x 18,840).
- 26-4 Relationship between 'chloride type' cell (cc) and accessory cell (ac). t, tubules (x 15,805).



27-1 Apical region of 'chloride type' cell of mullet pseudobranch. Note the thick layer of amorphous granular material (gm) adhering to the surface of the cell. ac, accessory cell; j, shallow junction (x 24,955).

27-2 Apical region of 'chloride type' cell of bass pseudobranch. The tubular network (t) of the 'chloride type' cell opens into the extracellular spaces between the 'chloride type' and accessory cells (ac) (small arrows). The two cells are joined by desmosomes (d). ep, epithelial cell; g, glycocalyx; j, shallow junctions, v, vesicles (x 27,560).



- 28-1 Apical region of 'chloride' cell of bass gill. The surface of the 'chloride' cell (cc) is thrown into numerous cytoplasmic projections (cp) and coated vesicles (cv) are found within the apical pit. ac, accessory cell; ep, epithelial cell; j, shallow junction, t, tubules; v, vesicles (x 18,096).
- 28-2 Accessory cell (ac) and 'chloride' cell (cc) in bass gill (x 14,805).



'Pseudobranch type' cells of the bass pseudobranch.

- 29-1 Region of fusion between the secondary lamellae (sl) of bass pseudobranch. pc, 'pseudobranch type' cells (Methylene blue x 344).
- 29-2 Light (pc) and dark 'pseudobranch type' cells (dpc) in the secondary lamellae (sl) of bass pseudobranch. bc, blood channel; ep, epithelial covering (Methylene blue x 532).
- 29-3 Relationship of the 'pseudobranch type' cells (pc) to the blood channel (bc) and epithelial cells (ep) of bass pseudobranch. bm, basement membrane; m, mitochondria, mu, mucous cell (x 4,725).



'Pseudobranch type' cells of the trout pseudobranch.

- 30-1 Organisation of secondary lamellae in trout pseudobranch. bc, blood channels; pc, 'pseudobranch type' cell; v, vacuole; vc, venous channel (Methylene blue x 207)
- 30-2 'Pseudobranch type' cells (pc) from two apposing lamellae separated by interstitial cells (i). bc, blood channel; v, vacuole (Methylene blue x 1,312)
- 30-3 Electron micrograph of region similar to 30-2. bc, blood channel; i, interstitial tissue; m, mitochondria; pc, 'pseudobranch type' cell; v, vacuole (x 4,410).



'Pseudobranch type' cells of trout pseudobranch.

- 31-1 Relationship between 'pseudobranch type' cells and blood channel (bc). The cells are packed with numerous mitochondria (m) surrounded by tubules (t). bm, basement membrane; nu, nucleus (x 8,010).
- 31-2 Base of 'pseudobranch type' cell separated from the blood channel (bc) by a basement membrane (bm). Note the characteristic arrangement of tubules (t) around the mitochondria (m). Bundles of cytoplasmic filaments (f) communicate with desmosomes (d) at the lateral membranes of two cells. g, mitochondrial granules (x 27,608).



Trout 'pseudobranch type' cells.

- 32-1 Obliquely cut section showing spirally coiled nature of tubules (t) around mitochondria (m) (x 28,080).
- 32-2 Circular and V shaped mitochondria (m) surrounded by the tubular network (t) (x 17,955).
- 32-3 The outer mitochondrial membranes and those of the tubules are closely associated and continuities between the two are sometimes seen (arrow). m, mitochondria; t, tubule (x 28,080).
- 32-4 Two centrioles (one diplosome).
  - 1. cross sectioned centricle;
  - 2. longitudinally sectioned centricle.
  - mt, triplets of fused microtubules (x 79,800).



- 33-1 Unusual organelle found in carp 'pseudobranch type'
  cell.
  c, cisterna; gr, dense granules; mp, membranous
  plate (x 131,110).
- 33-2 Unusual organelle associated with the nucleus (nu) of a carp 'pseudobranch type' cell. ne, nuclear envelope (x 9960).
- 33-3 Long chain of desmosomes (d) between two adjoining trout 'pseudobranch type' cells. f, bundle of filaments (x 28,600).



- 34-1 Large vacuole (V) in the apical region of trout 'pseudobranch type' cell. bm, basement membrane; f, bundle of cytoplasmic filaments (x 11,800).
- 34-2 The cytoplasmic vacuole (v) is surrounded by bundles of cytofilaments (f) and a band of dense material (dm) just below the surface of the vacuole membrane. r, ribosomes (x 22,620).
- 34-3 Small vesicles (ve) appear to be pinched off from the membrane surrounding the vacuole (v). dm, dense fibrous material; f, cytofilaments; r, ribosomes (x 37,700).



- 35-1 Light (1) and dark (2) 'pseudobranch type' cells in carp pseudobranch. dg, dense granules (probably glycogen); nu, nucleus (x 7610).
- 35-2 Light (1) and dark (2) 'pseudobranch type' cells in bass pseudobranch. Note the small dark nucleus (nü) in the dark cell (x 12,000).
- 35-3 Base of dark 'pseudobranch type' cell in bass pseudobranch. Note invaginations of the basement membrane (bm) (arrows). bc, blood channel; dg, dense granules (probably glycogen); fen, fenestrated endothelium of blood channel (capillary) (64,500).


Mucous cells.

- 36-1 Mucous cell in trout pseudobranch epithelium containing lightly staining mucous droplets (d) and basal Golgi complex (go). ep, epithelial cell (x 9,355).
- 36-2 Mucous cell in bass pseudobranch epithelium containing densely staining droplets (d) and basal nucleus (nu). ep, epithelial cell (x 14,000).
- 36-3 Mucous cell of bass pseudobranch stained with 15% phosphotungstic acid (P.T.A.) at pH 2. The droplets (d) show varying degrees of staining and often have a paler central region. The membranes (m) of the droplets are stained but the nucleus of the cell is unstained. ep, epithelial cell (x 17,640).



- 37-1 Light micrograph of rodlet cells (r) in the secondary lamella (sl) of bass pseudobranch. cl, 'chloride type' cell; m, mucous cell; ps, 'pseudobranch type' cell; su, surface of secondary lamella (Methylene blue x 1342).
- 37-2 Relationship between rodlet cells (r), mucous cells (m), 'chloride type' cells (cl) and epithelial cells (ep) in the secondary lamellae of bass pseudobranch. ap, apical pit; f, fibrous cytoplasmic border of rodlet cell; s, rodlet sac (x 5,200).



38-1 Immature rodlet cell (Type 1).

f, fibrous cytoplasmic border of mature rodlet cell; g, Golgi complex; ger, granular endoplasmic reticulum; i, membrane bound inclusion; m, mitochondrion; nu, nucleus; r, ribosomes; s, rodlet sac (x 20,161).

38-2 Developing rodlet cell (Type II).

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dc, dense central core; e, epithelial cell; f, fibrous cytoplasmic border; g, Golgi complex; ger, granular endoplasmic reticulum; r, ribosomes; v, vesicles; va, vacuole (x 15,020).



39-1 Immature rodlet cell (Type III). f, fibrous cytoplasmic border; g, Golgi complex; ger, granular endoplasmic reticulum; m, mitochondria; nu, nucleus; r, ribosomes; s, developing rodlet sac (x 20,020).

39-2 Mature rodlet cell (Type IV) at the surface of the pseudobranch epithelium. dc, dense central core; e, flattened epithelial cell; f, fibrous cytoplasmic border; m, mitochondria; s, rodlet sac; v, vesicles; va, vacuole (x 20,145).



- 40-1 Fibrous cytoplasmic border of mature rodlet cell. db, dense band beneath the plasma membrane (pm); dc, dense central core; mf, microfibrils; r, ribosomes; s, rodlet sac (x 42,890).
- 40-2 Apex of mature rodlet cell (ro) near the epithelium surface showing desmosome contact (d) with an epithelial cell (e). dc, dense central core; m, mitochondria; r, ribosomes (x 45,900).



- 41-1 Some rodlet cells contain large membranous vacuoles (V) or a dark fibrous border (fd).
  e, epithelial cell; s, rodlet sac (x 9263).
- 41-2 Rodlet cell with hyperchromatic nucleus (nu). f, fibrous cytoplasmic border; s, rodlet sac (x 18,900).
- 41-3 Possible rodlet cell opening in the interlamellar filament epithelium. cp, cytoplasmic projections; r, circular ridge (x 15,110).



Trout pseudobranch injected with microfil via the ventral aorta.

- 42-1 Efferent side. Two or more efferent filament arteries (efa) may be connected by short cross vessels between them (arrows). The efferent filament arteries are supplied by efferent lamellar arterioles (not visible here) from the secondary lamellae (sl). paa, primary afferent artery; pea, primary efferent artery (x 17).
- 42-2 Afferent side. The primary afferent artery (paa) supplies the afferent filament arteries (afa) which supply blood to the secondary lamellae capillaries (sl) via the afferent lamellar arterioles. A partly filled section of the pseudobranchial vein (pv) is visible. The filament arteries are sometimes connected by small cross vessels (arrows) (x 17).



Bass pseudobranch injected with microfil via the ventral aorta.

- 43-1 Afferent side of bass pseudobranch. The primary afferent artery (paa) supplies the afferent filament arteries (afa) which supply blood to the secondary lamella capillaries via afferent lamellar arterioles. Note the pseudobranchial vein (pv) leaving the organ close to the entry of the primary afferent artery. pea, primary efferent artery (x 14.5).
- 43-2 The lumen of the pseudobranchial vein (pv) is not uniform along its length. Near the extremities of the pseudobranch it is replaced by a series of smaller interconnecting vessels. afa, afferent filament arteries; paa, primary afferent artery; pea, primary efferent artery (x 18.5).



- 44-1 Pseudobranch arch of perch. paa, primary afferent artery; pea, primary efferent artery; ca, cartilage; v, venous channel. (H & E x 314).
- 44-2 Pseudobranch arch of rainbow trout. ep, epithelium; paa, primary afferent artery; pea, primary efferent artery; pv, pseudobranchial vein; sl, secondary lamellae; v, venous sinus behind the pseudobranch (Mallory's x 265).
- 44-3 Pseudobranch arch of carp. paa, primary afferent artery; pea, primary efferent artery; pv, pseudobranchial vein; sl, secondary lamellae; v, venous channel (Mallory's x 242).



- 45-1 The primary afferent artery (paa) of the bass pseudobranch is surrounded by numerous capillaries (cp) which it makes direct contact with by means of sphincter-like vessels supplying the tissues of the pseudobranch arch. The blood is eventually collected in veins which drain into the pseudobranchial vein (v). ca, cartilage (Mallory's x 342).
- 45-2 Numerous capillaries (cp) are packed around the primary afferent artery (paa) of the perch pseudobranch. They make frequent connections with the artery via short, sphincter-like vessels (arrows) (H & E x 385).
- 45-3 The primary efferent artery (pea) of perch pseudobranch is also surrounded by capillaries (cp) but these are part of the afferent nutritive system and have no connections to the efferent artery (H & E x 376).
- 45-4 Longitudinal section of trout primary afferent artery showing lining of endothelial cells (en) and muscular wall (m). rbc, red blood cells (x 3,370).



- 46-1 Bass pseudobranch arch showing position of the primary afferent artery (paa), primary efferent artery (pea), and large pseudobranchial vein (pv).
  c, cartilage (Mallory's x 225).
- 46-2 The pseudobranchial vein (pv) gives rise to the central venous sinus (cvs) in the core of the filament. Both vessels run close to the afferent filament artery (afa) and cartilage (c). sl, secondary lamellae (Mallory's x 176).
- 46-3 Extensions of the c.v.s. form venous channels (v) which run close to the cartilaginous ray of the filament (c) and the wall (w) of the afferent filament artery. Nerve bundles (n) are often seen close to these venous channels. lu, lumen of afferent filament artery (x 3,500).



Microfil injected pseudobranchs perfused via the ventral aorta.

- 47-1 Vessels (arrow) supplying the pseudobranchial vein (pv) of the bass which appear to arise from the afferent filament artery (afa). paa, primary afferent artery; pea, primary efferent artery (x 44.8).
- 47-2 Nutritive vessels (arrows) from bass primary afferent artery running parallel to the afferent filament artery (afa) (x 55).
- 47-3 Longitudinal and transverse nutritive vessels (arrows) running alongside the afferent filament arteries (afa) of trout pseudobranch. sl, secondary lamellae (x 75).



Microfil injected trout pseudobranch perfused via the primary efferent artery.

- 48-1 The secondary lamellae (sl) at the base of the organ are well filled. Note the direct connections between the primary efferent artery (pea) and secondary lamellae by means of short arterial vessels (black arrows). The efferent lamellar arterioles supplying the efferent filament artery (efa) may arise from two or three lamellae. Numerous nutritive vessels of the arteriovenous system run over the surface of the pseudobranch (white arrows) (x 45).
- 48-2 Nutritive vessels (white arrows) on the efferent side of the pseudobranch arise from the primary afferent artery (paa) (black arrows). efa, efferent filament artery; pea, primary efferent artery; sl, secondary lamellae (x 45).



- 49-1 Transverse section of bass pseudobranch filament. Extensions of the central venous sinus form venous channels (vc) outside the core of the filament. These often run close to the afferent filament artery (afa), afferent lamellar arterioles (ar) and small nutritive vessels (n).
  c, cartilage; bc, blood channel of secondary lamella; cc, 'chloride type' cell (Methylene blue x 614).
- 49-2 T.S. of trout pseudobranch filament and secondary lamellae (sl). The central venous sinus gives rise to sinus-like channels (vc) outside the core of the filament and running in the connective tissue between the afferent filament arteries (afa). c, cartilage (H & E x 162).
- 49-3 In the carp pseudobranch, a number of filamental sinuses may open into a large venous channel (vc) outside the core of the filament. sl, secondary lamellae (H & E x 124).



- 50-1 Transverse section of bass pseudobranch filament showing the connection between the efferent filament artery (efa) and central venous sinus (cvs) by means of an arterio-venous anastomose (ava). The endothelial cells of the ava protrude into the lumen of the efferent artery. The efferent artery is surrounded by venous channels(vc) which are extensions of the cvs. bc, blood channel of secondary lamella; pc, 'pseudobranch type' cell (Methylene blue x 614).
- 50-2 An arterio-venous anastomose (ava) is easily identified in paraffin sections by its conspicuously large, dark endothelial cells. efa, efferent filament artery; cvs, central venous sinus (Mallory's x 1248).



Arterio-venous anastomose between the efferent filament artery and central venous sinus of bass pseudobranch. cc, cover cells; en, endothelial cell; lu, lumen of efferent artery; g, dense membrane bound granules; in, intermediate cell; n, nerve bundle; v, vesicles; l, Type I endothelial cell; 2, Type II endothelial cell (x 12,250).



- 52-1 Type I A.V.A. endothelial cell. Note the highly filamentous nature of the cytoplasm and the arrangement of the filaments (f) into concentric whorls. Occasional dense granules (g) and a few vesicles (v) are also found. n, nucleus (x 26,950).
- 52-2 Junction (j) between Type I endothelial cell (l) and intermediate cell (in). m, mitochondrion; n, nucleus; v, vesicles (x 32,500).
- 52-3 Obliquely cut section of an A.V.A. showing the narrow lumen occluded by the intermediate cells (in), and the Type II endothelial cells (2) protruding into the central venous sinus (cvs). cc, cover cells; bl, basal lamina; n, nerve fibres (x 8,085).



- 53-1 Relationship between an A.V.A. and filament nerve bundles (n). en, endothelial cell of efferent filament artery; in, intermediate cells; lu, lumen of efferent filament artery; l, Type I endothelial cell (x 8450).
- 53-2 Naked, unmyelinated axons (un) run close to the endothelial cells of the A.V.A. which contain vesicles (v) and glycogen granules (g). n, nucleus (x 29,640).


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Microfil injected gills perfused via the ventral aorta.

- 54-1 Trout gill arch. Efferent side (x 5.25)
- 54-2 Perch gill arch. Efferent side (x 6.6).
- 54-3 Departure of the efferent filament arteries (efa) from the efferent branchial artery (eba) in the trout gill. Note the rich capillary network around the base of the efferent filament arteries and the two small arteries which run alongside each filament artery (arrows). sl, secondary lamellae (x 42).



Microfil injected gills perfused via the ventral aorta.

- 55-1 Transverse section of trout gill arch and attached filaments. Note the enlargements, or blebs (b), on the afferent filament artery (afa). Up to these swellings the opposite filaments are joined together by adductor muscles which possess a rich vascularisation (vm). Part of the central venous sinus (cvs) can be seen where the secondary lamellae (sl) have been removed. aba, afferent branchial artery; efa, efferent filament artery (x 40).
- 55-2 Inside view (afferent side) of the filaments in trout gill. Note the 'blebs' (b) on the afferent filament artery (afa) and the rich vascularisation (vm) of the adductor muscles in the septal region (x 40).
- 55-3 Base of the efferent filament arteries (efa) proximal to the efferent branchial artery (eba) of the perch gill. Note the interconnecting vascular network between the filament bases, and the small arteries running alongside the filament arteries (arrows) (x 55).



- 56-1 Inside view (afferent side) of the base of trout filaments proximal to the gill arch. The afferent filament arteries (afa) arise from the efferent branchial artery (aba) which is surrounded by veins (v) collecting blood from the arterio-venous system. eba, efferent branchial artery (x 40).
- 56-2 Detailed frontal view of the base of the efferent filament arteries (efa) of the trout showing the rich capillary network (arrows) surrounding the vessels near their connection to the efferent branchial artery (x 68).
- 56-3 Distal region of trout gill filaments (efferent side) showing the short extensions of the C.V.S. (arrows) which surround the efferent filament artery (efa) (x 64).
- 56-4 Sagittal view of the central venous sinus (cvs) in two incompletely injected filaments of bass gill. Note the simple sac-like structure of the C.V.S. and its proximity to the efferent filament artery (efa) (x 102).

ate efo eba A efc

Microfil injected choroid rete mirabile.

57-1 The choroid rete mirabile is large horseshoe-shaped capillary network. c, capillary network; oa, ophthalmic artery; ov, ophthalmic vein; ovs, ophthalmic venous sinus (x 23).

57-2 Detailed view of the capillary network (c) of the choroid rete mirabile showing its highly branched nature. ovs, ophthalmic venous sinus (x 45).





- 58-1 Branch of trout pseudobranch nerve (n) running in the adipose tissue (ad) of the pseudobranch arch. paa, primary afferent artery (Mallory's x 174).
- 58-2 Branches of trout pseudobranch nerve (n) surrounded by fat cells (fc), small nutrient arteries (ar) and venous channels (v) (Methylene blue x 345).



Methylene blue sections of trout pseudobranch nerve.

- 59-1 Transverse section of pseudobranch nerve cut parallel with the primary efferent artery (x 573).
- 59-2 Separation of the nerve into smaller bundles (x 448).
- 59-3 Separation of the nerve into bundles (b) containing mainly small fibres (sf) or large fibres (lf) (x 368). n, nerve fibre; m, myelin sheath.



- 60-1 Transverse section of trout pseudobranch nerve (x 3640).
- 60-2 Detailed view of pseudobranch nerve fibres (x 6,160). a, axoplasm of myelinated fibres; co, collagen fibres; e, endoneurium; my, myelin sheath; n, nucleus of Schwann cell; p,perineurium; un, unmyelinated nerve fibres.



- 61-1 Longitudinal section of unmyelinated (un) and myelinated (my) fibres of trout pseudobranch nerve. co, collagen (x 4,760).
- 61-2 Detailed view of a longitudinally sectioned myelinated fibre. co, collagen; m, mitochondrion; my, myelin sheath; nf, neurofilaments; nt, neurotubules (x 47,600).
- 61-3 Detailed view of transversely sectioned unmyelinated fibres. m, mitochondrion; nf, neurofilaments; nt, neurotubules (x 47,600).



Primary nerve plexus. Cajal silver impregnation method.

- 62-1 Part of primary plexus in the bass pseudobranch arch, which supplies the secondary plexus in the axis of each filament (x 573).
- 62-2 Dense innervation of the primary afferent artery in the bass pseudobranch (x 448).
- 62-3 Collection of nerve cell bodies of the primary plexus near the base of a trout pseudobranch filament ( x 1,400).
- 62-4 Two closely associated unipolar neurons (n) of the primary plexus in trout pseudobranch. Note the long nerve process (np) arising from one of the neurons (x 1280).



- 63-1 Bundle of nerve fibres of the primary plexus containing unmyelinated (un) and myelinated fibres (my). co, collagen fibres (x 9750).
- 63-2 Unmyelinated nerve fibres (un) of the primary plexus surrounded by Schwann cell cytoplasm (s). Note the axoplasm of the larger fibres (l) which may contain a more extensive agranular endoplasmic reticulum, more mitochondria (m) and large numbers of neurofilaments. co, collagen fibres; n, nucleus of Schwann cell (x 7,610).
- 63-3 Nerve cell bodies in the primary plexus, showing the characteristic stacks of granular endoplasmic reticulum (Nissl substance, ni). co, collagen; ly, small dense lysosome; n, nucleus; nu, nucleolus; p, nerve cell process (x 5400).



- 64-1 Small nerve cell body of the primary plexus with closely associated nerve cell processes (p) and a bundle of unmyelinated fibres (un). g, Golgi complex; ger, granular endoplasmic reticulum; ly, lysosome (x 11,200).
- 64-2 Axo-somatic synapse (sy) between an axon bouton (a) and a nerve cell body (n). The bouton contains a mitochondrion and an accumulation of vesicles at the pre-synaptic membrane of the axon (1). Note the thickening of the perikaryon post-synaptic membrane (2). r, ribosomes (x 44,200).
- 64-3 Small group of naked unmyelinated nerve fibres (un) near a nerve cell body. A myelinated fibre is also present and contains neurofilaments (nf) and neurotubules (nt) (x 27,500).
- 64-4 Naked unmyelinated fibres of various sizes near a nerve cell body. nt, neurotubules (x 27,500).



- 65-1 Primary afferent artery in the trout pseudobranch arch innervated by small bundles of nerve fibres (nf) (x 416) (Cajal silver impregnation method).
- 65-2 Region of trout primary efferent artery innervated by short, thick nerve processes (np) polarised towards the lumen (lu) of the vessel (x 1120) (Cajal method).



- 66-1 Small bundle of fibres in the adventitia of trout primary afferent artery. A group of unmyelinated fibres (un) is often accompanied by a myelinated fibre (my). lu, lumen of small nutritive artery; en, endothelial cell (x 4,760).
- 66-2 Unmyelinated nerve fibres (un) in the adventitia of the trout primary afferent artery containing an extensive agranular endoplasmic reticulum (er) of tubular and vesicular profiles. An occasional dense core vesicle is also seen (v). co, collagen fibres (x 17,955).
- 66-3 Unmyelinated nerve fibres (un) in the adventitia of trout primary afferent artery. The fibres contain a variety of clear vesicles (v), dense core vesicles (dcv), mitochondria (m), glycogen particles (g) and tubular profiles of endoplasmic reticulum (t). co, collagen fibres (x 30,840).



- 67-1 Nerve fibre containing clear vesicles (v), dense core vesicles (dcv) and glycogen granules (g) close to the smooth muscle cells (mu) of trout primary afferent artery wall. f, myofilaments (x 29,850).
- 67-2 Bundle of unmyelinated fibres (un) in the adventitia
  of trout primary efferent artery.
  co, collagen fibres; e, elastic tissue; en, endothelial
  cell; lu, lumen of efferent artery (x 8,620).
- 67-3 Small neuron (n) adjacent to the adventitia of the primary efferent artery of the trout pseudobranch. co, collagen fibres; e, elastic tissue; en, endothelial cell; lu, lumen of efferent artery; un, unmyelinated nerve fibre (x 4,400).
- 67-4 Small bundle of unmyelinated fibres (un) close to a nutritional artery. co, collagen fibres; en, endothelial cell; lu, lumen of artery; m, muscle cell (x 9,330).



- 68-1 Nerve fibres (nf) of the secondary plexus in the axis of trout pseudobranch filament. sl, secondary lamella (x 1580) (Cajal method).
- 68-2 Bundle of unmyelinated nerve fibres (un) running in the central compartment of trout pseudobranch filament. bl, basal lamina (x 9,100).
- 68-3 Unmyelinated nerve fibres (un) in the central compartment of trout pseudobranch filament. n, nucleus of Schwann cell (x 9,100).
- 68-4 Small bundle of naked unmyelinated fibres (un) in the central compartment of mullet pseudobranch filament. co. collagen fibres;; bl, basal lamina (x 19,500).



- 69-1 Bundle of unmyelinated nerve fibres (un) running between the efferent filament artery and c.v.s. of bass pseudobranch filament. en, endothelial cell; lu, lumen of efferent artery; n, nucleus of Schwann cell; s, Schwann cell cytoplasm; sm, smooth muscle of artery wall (x 7,743).
- 69-2 Detailed view of nerve bundle in 69-1 showing unmyelinated fibres (un) and Schwann cell cytoplasm rich in granular endoplasmic reticulum (ger), Golgi membranes (go) and free ribosomes (r) (x 14,500).
- 69-3 Large unmyelinated fibres (un) in the central compartment around the outside of bass efferent filament artery. Note the clear cytoplasm containing mitochondria (m) and occasional glycogen particles (g) (x 30,160).
- 69-4 Large unmyelinated fibre from the same region as those in 69-3. The cytoplasm is filled with small mitochondria (m), dense lamellar bodies (lb), lamellar whorls (lw) and tubular (t) and vesicular (v) elements (x 30,160).



- 70-1 Bundles (b) of unmyelinated nerve fibres (un) at the base of trout pseudobranch secondary lamellae. lu, lumen of marginal vessel (x 8,010).
- 70-2 Bundle of unmyelinated fibres (un) penetrating the interstitial tissue at the base of a secondary lamella (x 9,100).
- 70-3 Detailed view of unmyelinated fibres at the base of a secondary lamella. nf, neurofilaments; nt, neurotubules (x 27,700).





- 71-1 Nerve fibre (nf) in the secondary plexus of trout pseudobranch containing mitochondria (m), dense core vesicles (dcv), clear vesicles (v) and neurotubules (nt) (x 14,000).
- 71-2 Nerve cell body (n) with a number of dense lysosomes (ly). nu, nucleus; un, unmyelinated fibre (x 6,160).
- 71-3 Pale nerve cell body (n) and large nerve cell process (cp) containing a prominent Golgi complex (g), mitochondria (m) and small lysosomes (ly) (x 11,200).


- 72-1 Small, ovoid nerve cell body with large pale nucleus (nu) and small numbers of mitochondria (m) and lysosomes (ly). Note the small tubular and vesicular elements containing an electron dense core (arrows). ger, granular endoplasmic reticulum (x 12,500).
- 72-2 Specialized regions of contact between neighbouring neurons are made by gap junctions (j). nu, nucleus; un, unmyelinated fibres (x 28,050).
- 72-3 Small irregularly shaped nerve cell body surrounded by large pale cell processes (cp) and unmyelinated fibres (un). ly, lysosome; m, mitochondria; nu, nucleus (x 14,000).



- 73-1 Possible synapse between two nerve fibres. One fibre contains clear (v) and dense core vesicles (dcv) while the other contains mitochondria (m) (x 29,640).
- 73-2 Small bundle of unmyelinated nerve fibres in the adventitia of the afferent filament artery in trout pseudobranch. Some fibres contain vesicular (ver) and tubular (ter) profiles of agranular endoplasmic reticulum. m, mitochondria; g, glycogen; co, collagen (x 29,640).
- 73-3 Small unmyelinated nerve fibres containing clear vesicles (v) and mitochondria (m) in the adventitia of the afferent filament of mullet pseudobranch. co, collagen; el, elastic tissue; en endothelial cell; lu, lumen of blood vessel; mu, muscle cell (x 19,500).



- 74-1 Small group of naked unmyelinated fibres (un) in the adventitia of the mullet efferent filament artery. co, collagen; v, clear vesicles (x 27,500).
- 74-2 Small group of naked unmyelinated fibres (un) close to muscle cells (mu) around mullet afferent filament artery. co, collagen; v, clear vesicles (x 27,500).
- 74-3 Bundle of unmyelinated nerve fibres (un) close to the muscle layer (mu) surrounding a small nutritional artery. en, endothelial cell; lu, lumen of blood vessel (x 22,100).



- 75-1 Two small neurons (nl and n2) in the secondary lamella of trout pseudobranch (Bielchowsky method x 820).
- 75-2 Nerve fibres of the terminal plexus in the secondary lamella of trout pseudobranch (Bielchowsky method x 1,030).
- 75-3 Nerve fibre in the secondary lamella of bass pseudobranch ending in a bouton-like swelling (b) and possessing a number of fibre expansions (e) (Cajal method x 1664).
- 75-4 Bundle of unmyelinated nerve fibres (un) at the base of the secondary lamellae in trout pseudobranch. The fibres contain a variety of organelles which may include a network of agranular endoplasmic reticulum (er), dense bodies (db), glycogen particles (g), clear and dense core vesicles (v) and neurotubules (nt).

n, nucleus of interstitial tissue (i) (x 21,560).



- 76-1 Bundle of unmyelinated fibres (un) penetrating the interstitial tissue between the pseudobranch cells (pc) at the base of the secondary lamellae in trout pseudobranch. The fibres are in close contact with large vacuolated regions(V) and large, pale cytoplasmic expansions containing a variety of membranous structures (x 4,760).
- 76-2 Thick and thin appearance of a nerve fibres (nf) with two varicosities containing clear and dense core vesicles (v) and mitochondria (m). Note its position adjacent to a 'pseudobranch type' cell (pc) and surrounded by interstitial tissue (i) (x 19,890).
- 76-3 Nerve fibre expansion containing a variety of vesicular (v) and tubular (t) elements of varying electron density, glycogen (g), mitochondria (m) and neurotubules (nt) Trout pseudobranch (x 21,450).



- 77-1 Two nerve fibre expansions in the terminal plexus of trout pseudobranch. Note the accumulation of vesicles (v) and small mitochondria (sm) in one fibre and the collection of small mitochondria, large mitochondria (m) and whorled lamellar (lw) structures in the other. nf, neurofilaments (x 130,000).
- 77-2 Nerve fibre expansions in the secondary lamellae of trout pseudobranch containing an accumulation of small dense mitochondria (m), whorled lamellar structures (lw) and glycogen particles (g). i, interstitial tissue; pc, 'pseudobranch type' cell (x 28,600).



- 78-1 Nerve fibres in the terminal plexus of trout pseudobranch, containing glycogen particles (g), clear vesicles (v), mitochondria (m) and neurotubules (nt) (x 37,440).
- 78-2 Possible nerve ending in the terminal plexus of trout pseudobranch containing mitochondria (m) and clear (v) and dense core vesicles (dcv) (x 27,560).
- 78-3 Small bundle of naked axons in the terminal plexus of trout pseudobranch. nt, neurotubules; v, clear vesicles; i, interstitial tissue (x 23,100).
- 78-4 Nerve fibre expansion in the terminal plexus of carp pseudobranch, containing mainly small mitochondria (m) and glycogen particles (g) (x 71,550).



- 79-1 Nerve fibre expansion containing an accumulation of small mitochondria (sm) and whorled lamellar structures (lw). Trout pseudobranch. i, interstitial tissue (x 35,815).
- 79-2 Nerve fibre expansions with a variety of vesicles (v), small mitochondria (sm), lamellar whorls (lw), tubular endoplasmic reticulum (ter) and membranous profiles (me). nt, neurotubules.Trout pseudobranch (x 37,225).
- 79-3 Nerve fibre containing small mitochondria (sm) and a collection of vesicular (v) and tubular (t) elements. Trout pseudobranch (x 71,550).
- 79-4 Nerve fibre expansions of trout terminal plexus. One fibre contains a network of vesicular endoplasmic reticulum (ver) and vacuoles (va). g, glycogen; m, mitochondria; nt, neurotubules; v, clear vesicles (x 39,000).



- 80-1 Synapse (sy) between a nerve fibre and trout 'pseudobranch type' cell (pc). The nerve fibre contains an accumulation of clear vesicles (v), occasional dense core vesicles (dcv) and mitochondria (m). The membrane of the nerve ending bears a number of dense regions (white arrows) while that of the 'pseudobranch type' cell shows a slight uniform thickening (black arrow) (x 70,200).
- 80-2 Possible synapse (sy) between a nerve ending and trout 'pseudobranch type' cell. g, glycogen; dcv, dense core vesicle; V, clear vesicles; nt, neurotubules; va, vacuole (x 27,560).
- 80-3 Nerve ending adjacent to a trout 'pseudobranch type' cell with a dense membranous region (white arrow), accumulation of clear vesicles (v), and a vesicle in a membranous cleft of the ending (black arrow). g, glycogen; nt, neurotubules (x 71,550).



- 81-1 Trout pseudobranch nerve, 7 days after denervation. Some distortion and fragmentation (f) of the myelin sheaths is evident. a, axoplasm; n, nucleus of Schwann cell; un, unmyelinated fibres (x 3,240).
- 81-2 Trout pseudobranch nerve, 7 days after denervation. Nerve fibre showing fragmentation (f) of the myelin sheath (my) and vacuolation (v) of the axoplasm (a). co, collagen (x 4860).
- 81-3 Trout pseudobranch nerve, 7 days after denervation.
  Axoplasm (a) of myelinated nerve fibre containing a mass of degenerating myelin (d).
  co, collagen; un, unmyelinated fibres (x 16,065).



Trout pseudobranch nerve, 28 days after denervation.

82-1 Most of myelin sheaths have disappeared although occassional profiles of disintegrating myelin (dm) are still seen. Note the proliferation of Schwann cells (sc).

n, nucleus of Schwann cell (x 3740).

- 82-2 The Schwann cells (sc) show various stages of innervation by regenerating axons (a). n, nucleus (x 6800).
- 82-3 Regenerating axons (a) surrounded by highly folded membranes of the Schwann cells. Note the presence of small electron dense droplets (d) in the axons and Schwann cell cytoplasm. n, nucleus (x 19,800).



- 83-1 Trout pseudobranch nerve, 56 days after denervation. There is an increase in the diameter of the axons (a) and remyelination (my) of some. co, collagen; Sc, Schwann cell; n, nucleus of Schwann cell (x 6800).
- 83-2 Trout pseudobranch nerve, 70 days after denervation. More axons (a) are myelinated (my) although only the largest fibres are undergoing this process. co, collagen; nu, nucleus of Schwann cell (x 8,500).



Trout pseudobranch nerve, 105 days after denervation.

- 84-1 The regeneration process is essentially complete although the number of myelinated fibres is significantly less than in normal nerve. Numerous unmyelinated axons (un) are present. a, axon; n, nucleus of Schwann cell (x 4,800).
- 84-2 Myelinated axons (a) and unmyelinated fibres (un) surrounded by a large number of collagen fibres (co). n, nucleus of Schwann cell (sc). (x 12,420).
- 84-3 Some Schwann cells (sc) still contain degenerate myelin debris (d) and show no sign of reinnervation (x 11,900).



- 85-1 Large neuron of trout primary plexus, 7 days after denervation. The granular endoplasmic reticulum (ger) is disorganised and the mitochondria are vacuolated (vm). ly, lysosome; co, collagen; un, unmyelinated fibres; va, vacuolated axoplasm of a myelinated fibre (x 8415).
- 85-2 Neuron of trout primary plexus, 7 days after denervation. The granular endoplasmic reticulum (ger) is less obviously affected although the mitochondria are vacuolated (vm). An axon (a) can be seen arising from the neuron.

un, unmyelinated fibres (x 5,920).



- 86-1 Neuron of trout primary plexus showing folding (f) of the nucleus (nu), 7 days after denervation. ger, granular endoplasmic reticulum; j, gap junction; ly, lysosome; m, mitochondria (x 17,325).
- 86-2 Type 2 neuron in the secondary plexus showing a proliferation of lysosomes (ly), 7 days after denervation. go, Golgi complex; m, mitochondria; nu, nucleus (x 11,200).
- 86-3 Large neuron of trout primary plexus, 4 weeks after denervation. Note the disappearance of ribosomes, highly vesiculated nature of the Golgi complex (go) and the appearance of dense droplets (d). The cisternae of the endoplasmic reticulum (er) are arranged in an orderly parallel manner. Regenerating axon sprouts (a) can be seen close by. n, nucleus of neuron; co, collagen (x 9000).



- 87-1 Large neuron of trout primary plexus, 105 days after denervation. The nucleus (n) appears uniformly electron dense with no sign of distortion, but the mitochondria are vacuolated (vm) and the granular endoplasmic reticulum (ger) still appears disorganised. There are large numbers of polyribosome rosettes (r). go, Golgi apparatus; ly, lysosome (x 10,400).
- 87-2 Neuron of trout primary plexus, 105 days after denervation, showing organisation of the granular endoplasmic reticulum (ger) into parallel cisternae. sc, Schwann cell nucleus; un, unmyelinated fibres; vm, vacuolated mitochondria (x 8,000).



- 88-1 Type 2 neuron of trout secondary plexus, 105 days after denervation. Note the pale cytoplasm containing free ribosomes (r), vesicular (ve) and tubular (t) elements, vacuoles (va), small dense bodies (db) and lysosome-like bodies containing stacks of membranous material (me). The nuclear envelope (ne) of the nucleus (n) is distended (x 26,000).
- 88-2 Mixed nerve bundle of trout secondary plexus, 7 days after denervation. The unmyelinated fibres (un) appear unaffected but the myelin sheaths of myelinated fibres show signs of folding and disintegration (d) (x 12,250).



Terminal plexus of trout pseudobranch, 7 days after denervation.

- 89-1 Unmyelinated fibres in the terminal plexus of trout pseudobranch, containing irregular membranous profiles (mp). ve, vesicles (x 17,640).
- 89-2 Unmyelinated fibre in the interstitial tissue (i) of trout pseudobranch. The fibre is vacuolated (va) and there appears to be clumping (c) of some vesicles. f, bundle of fibres (x 30,250).
- 89-3 Unmyelinated fibres showing vacuolation (va) and apparent disorganisation of neurofilaments (f). co, collagen; m, mitochondria (x 8,910).
- 89-4 Unmyelinated fibres showing clumping of small vesicles (c), vacuolation (va) and formation of large membranous vesicles (ve) (x 16,380).


- 90-1 Nerve fibre expansion in the secondary plexus of trout pseudobranch, 7 days after denervation. The expansion is filled with dense lysosomal bodies (db) which arise from degenerating mitochondria (x 19,800).
- 90-2 Nerve fibre of trout terminal plexus, 28 days after denervation. The fibre contains dense lamellar bodies (1b) and shows some breakdown of its plasma membrane (arrows) (x 28,080).
- 90-3 Nerve fibre of trout terminal plexus, 21 days after denervation. Note the appearance of a large myelin figure (mf) which may arise from autolysed mitochondria (x 39,000).
- 90-4 'Pseudobranch type' cell (pc) 21 days after denervation, containing a variety of myelin figures (mf) and lipid droplets (1) (x 28,600).



- 91-1 Unmyelinated fibres of trout secondary plexus, 28 days after denervation. There appears to be some fragmentation and disorganisation of neurotubules (nt) and neurofilaments (nf) (x 16,380).
- 91-2 Unmyelinated fibres of trout secondary plexus, 70 days after denervation. Some of the fibres contain vacuolated areas (va) and there appears to be breakdown of neurotubules and neurofilaments. m, mitochondria (x 10,400).
- 91-3 Unmyelinated nerve fibres close to a trout pseudobranch cell (pc) 105 days after denervation. The nerve fibres appear unaffected but the 'pseudobranch type' cell appears abnormal with the formation of myelin figures (mf) and breakdown of the plasma membrane (arrow). i, interstitial tissue; nf, neurofilaments; ve, vesicles (x 28,600).



- 92-1 Large unmyelinated nerve process close to a large neuron of trout primary plexus. The nerve process appears abnormal with large vacuolated areas (va), large membranous vesicles (v) and distorted neuro-filaments (nf). The granular endoplasmic reticulum (ger) of the neuron is distended and the mitochondria (m) are vacuolated.
  ly, lysosome. 70 days after denervation (x 16,380).
- 92-2 Abnormal appearance of some fibres in trout terminal plexus 105 days after denervation. The fibres may be devoid of most organelles apart from occassional mitochondria (m) and vesicles, or they may contain a disrupted network of agranular endoplasmic reticulum (er). (x 20,280).
- 92-3 Synapse (sy) between nerve fibre (nf) and trout 'pseudobranch type' cell (pc) 105 days after denervation (x 20,280).



- 93-1 Fibres of trout terminal plexus 70 days after denervation. The fibres may contain large vacuoles (va) or show complete dissolution of the fibre cytoplasm. One fibre contains a collection of membranous structures, dense bodies (db) and glycogen particles. i, interstitial tissue; pc, 'pseudobranch type' cell (x 11,138).
- 93-2 Some fibres of trout terminal plexus, 105 days after denervation, are very pale (pf) and appear to have lost most organelles apart from a few small dense bodies (db), mitochondria and myelin figures (mf). i, interstitial tissue; pc, 'pseudobranch type' cell (x 8,900).



- 94-1 P.A.S. test, bass pseudobranch type cell. The mucous cells (m) and rodlet cells (rc) are strongly stained. The 'chloride type' cells are stained tightly but the 'pseudobranch type' cells show little or no reaction. efa, efferent filament artery; sl, secondary lamella (x 189).
- 94-2 Rodlet cells in bass pseudobranch stained by PAS test. Counterstained with H & E. pc, 'pseudobranch type' cell (x 333).
- 94-3 Bass pseudobranch secondary lamellae stained by Nitro-B.T. method for succinic dehydrogenase. Note the strong staining reaction of the 'pseudobranch type' cells and the weaker reaction of the 'chloride type' cells (cc) (x 214).



- 95-1 Localisation of chloride ions in bass 'pseudobranch type' cells (pc). Clumps of precipitate (p) can be seen adhering to the inner and outer surface of the apical membrane as well as to adjacent nerve fibres (nf) (x 10,800).
- 95-2 Localisation of sodium ions in mullet 'pseudobranch type' cells (pc). A considerable amount of precipitate is seen in the cytoplasm of these cells (x 10,800).
- 95-3 Localisation of sodium ions in mullet 'pseudobranch type' cell. A very fine precipitate can be seen in the heterochromatin region of the nucleus (n). Larger particles are scattered throughout the cytoplasm. m, mitochondria; t, tubule (x 29,700).
- 95-4 'Dark' 'pseudobranch type' cell of mullet pseudobranch showing staining reaction of glycogen particles (g) to the potassium pyroantinomate method for sodium ions. t, tubules (x 45,900).



Localisation of alkaline phosphatase activity in trout pseudobranch.

- 96-1 Reaction product (p) is seen as a fine granular deposit in the tubules of the pseudobranch type cell. m, mitochondria (x 28,000).
- 96-2 Reaction product (p) is localised in the centre of the tubules, apparently within the border of an intracisternal tubular system inside the main tubules (t). m, mitochondria (x 36,000).
- 96-3 Control section of trout pseudobranch incubated without sodium-B-glycerophosphate. m, mitochondria; t, tubules (x 22,000).



- 97-1 Localisation of carbonic anhydrase activity in trout 'pseudobranch type' cell (pc). The reaction product (p) is associated with the tubular system (t) near the basement membrane (bm) (Unstained x 66,000).
- 97-2 Localisation of carbonic anhydrase activity in trout 'pseudobranch type' cell (pc). bm, basement membrane; p, reaction product; t, tubular system (Unstained x 66,000).
- 97-3 The reaction product (p) appears to be associated with the outer membranes of the tubules (t). bm, basement membrane (Unstained x 135,000).





- 98-1 Localisation of adenosine triphosphatase activity in bass pseudobranch. Most reaction product is localised at the anti-vascular pole of the 'pseudobranch type' cells (pc) in the intercellular spaces of the lateral and apical membranes. i, interstitial tissue; m, mitochondria; n, nucleus; t, tubules (unstained x 36,400).
- 98-2 Carp 'pseudobranch type' cell stained with osmium tetroxide. Note the dense staining reaction of the nuclear envelope (ne), Golgi apparatus (go) and the large homogenous droplets (d). bc, blood channel; bm, basement membrane; n, nucleus; m, mitochondria; va, vacuole (x 7,464).



Carp 'pseudobranch type' cell stained with osmium tetroxide.

- 99-1 Dense staining reaction of Golgi complex (go), large homogenous droplets (d) and vacuoles (x 20,526).
- 99-2 Deposits of osmium black in the tubulovesicular cristae of mitochondria (m) (x 34,000).
- 99-3 Basal region of 'dark pseudobranch type' cells showing heavy deposits amongst the tubular system. bm, basement membrane (x 31,722).
- 99-4 Staining of possible multivesicular bodies (mv.) and the unusual organelle associated with the nucleus (n) of carp 'pseudobranch type' cells (arrows) (x 20,526).



Localisation of chloride ions in bass pseudobranch.

- 100-1 Chloride reaction product can be seen outlining the apical pits (ap) of the 'chloride type' cells (cc). bc, blood channel (Methylene blue x 1120).
- 100-2 Apical pit (ap) of 'chloride type' cell outlined by chloride reaction product. A small hole has appeared in the embedding matrix adjacent to the deposit. (x 14,000).
- 100-3 The clumps of reaction product are localised mainly on the cytoplasmic side of the apical membrane. ap, apical pit (x 21,840).
- 100-4 Clumps of reaction product associated with the shallow apical junctions (j) between the accessory (ac) and 'chloride type' cells. ap, apical pit (x 17,640).



- 101-1 Localisation of sodium ions in mullet 'chloride type'
   cell. A large amount of precipitate is found in the
   cytoplasm near the base of the cell around the network
   of narrow saccules (s).
   m, mitochondria; t, tubular network (x 17,010).
- 101-2 Apical region of mullet 'chloride type' cell showing
  precipitate in the cytoplasm and large vacuoles.
  ap, apical pit; v, vesicles (x 24,800).
- 101-3 Nucleus (n) of mullet 'chloride type' cell showing a
   deposit of very fine particles, particularly associated
   with the heterochromatin.
   m. mitochondria; t, tubules (x 35,100).
- 101-4 Localisation of alkaline phosphatase activity in the bass pseudobranch. Small amounts of precipitate (p) are associated with the lateral membranes (lm) in the apical region of the 'chloride type' cell. ap, apical pit (x 22,000).



- 102-1 Mucous cell stained with 1% P.T.A. (pH 0.3). The majority of globules (g) are lightly stained with a densely staining central region (d) (x 29,700).
- 102-2 Rodlet cell stained with 1% P.T.A. (ph 0.3). The sacs (rs) are densely stained but the central core (c) remains unstained (x 29,700).



- 103-1 Mucous cell (mu) and rodlet cell (rc) after staining with 15% PTA (pH 2). Mucous cell globules and membranes are stained whilst rodlet cell sacs and fibrous border remain unstained (x 16,225).
- 103-2 Carbonic anhydrase activity in bass rodlet cell. Reaction product is localised in the intercellular spaces of the lateral membranes in the apical region,
  and associated with the mitochondrial membranes (m). dc, dense core; ep, epithelial cell; rs, rodlet sac; w, fibrous wall of rodlet cell (x 32,500).



- 104-1 Carbonic anhydrase activity in bass rodlet cell. Precipitate is found outlining the membranes of the apical vesicles (v) as well as the mitochondria (m). ep, epithelial cell (x42,830).
- 104-2 Localisation of sodium ions in epithelial cells (ep) of mullet pseudobranch. Small amounts of precipitate are found in the cytoplasm and the surface membrane (sm) of one cell is densely stained (x 13,400).
- 104-3 Epithelial cell (ep) of mullet pseudobranch stained for sodium ions. The cytoplasm contains a small amount of precipitate while the nucleus (n) is covered with a precipitate of very fine particles (x 13,400).
- 104-4 Red blood cell (rbc) in mullet pseudobranch. The nucleus (n) is densely stained by the pyroantinomate method for sodium ions. bm, basement membrane (x 21,060).



- 105-1 'Chloride type' cell of the pseudobranch of bass kept in 0.34 % salinity. The apical region of the cell contains numerous vesicles (v), and is covered by a layer of epithelial cells (ep). ac, accessory cell; m, mitochondria; t, tubules (x 14,950).
- 105-2 'Chloride type' cell of the pseudobranch of bass kept in 0.34% salinity. Note the absence of saccules usually associated with the base of the cell. bc, blood channel; m, mitochondria; t, tubules (x 28,600).



- 106-1 'Chloride type' cell of bass pseudobranch after 5 hours
   exposure to 0.34% salinity. The cisternae of the
   granular endoplasmic reticulum (ger) have become very
   dilated.
   m, mitochondria (x 11,840).
- 106-2 Dense, lysosome-like body found in the epithelium of bass pseudobranch after 5 hours exposure to 0.34‰ salinity (x 11,750).
- 106-3 'Chloride type' cell of bass pseudobranch after 24 hours
  exposure to 0.34‰ salinity. Note the appearance of
  dense membrane bound inclusions (d) within the cell.
  (x 11,200).



'Chloride type' cells of bass pseudobranch exposed to 0.34% salinity.

- 107-1 Degenerating 'chloride type' cell after 36 hours exposure. The cell has a much darker cytoplasm than normal cells and the tubular network is thrown into lamellar whorls in the basal and lateral regions (x 16,095).
- 107-2 Degenerating 'chloride type' cell after 42 hours exposure. Many of the mitochondria are vacuolated and the cell contains large numbers of vacuoles, and a dense cytoplasm and nucleus (x 8613).
- 107-3 Degenerating 'chloride type' cell after 48 hours exposure. The cell is highly irregular in shape with a hyperchromatic nucleus (n) and a dense vacuolated cytoplasm (x 20,790).


'Pseudobranch type' cells of bass pseudobranch exposed to 0.34% salinity.

- 108-1 'Pseudobranch type' cell after 24 hours exposure. Note the appearance of spherical, electron dense inclusions (d) at the anti-vascular pole of the cell.
  bm, basement membrane; g, glycogen granules;
  t, tubules (x 26,300).
- 108-2 'Pseudobranch type' cell after 24 hours exposure, containing a large clear vacuole (v). bm, basement membrane; d, electron dense inclusions (x 7476).
- 108-3 Electron dense inclusion (d) aggregated very close to the apical membrane of a 'pseudobranch type' cell after 36 hours exposure. m, mitochondria (x 26,526).



- 109-1 'Chloride type' cell of smelt pseudobranch in fish
  adapted to freshwater. The apical membrane (am) of
  the cell protrudes beyond the edges of the epithelial
  cells.
  m, mitochondria; n, nucleus; s, saccules;
  t, tubules (x 11,400).
- 109-2 'Pseudobranch type' cell of trout adapted to 60%. salinity. The tubules show a random, disorganised appearance with disruption of the basotubular system communicating with the blood channel. bm, basement membrane; m, mitochondria; t, tubules; v, vacuole (x 18,920).



- 110-1 Desmosomes (d) between 'pseudobranch type' cells of trout adapted to 42% salinity (x 59,400).
- 110-2 'Pseudobranch type' cell of trout adapted to 42%. salinity. Note the highly branched, random appearance of the tubules (t). bm, basement membrane; m, mitochondria (x 35,100).
- 110-3 Possible rodlet cell (rc) in bass pseudobranch after 5 hours exposure to 0.34‰ salinity (x 6,240).
- 110-4 Possible degenerating rodlet cell (rc) in bass pseudobranch after 5 hours exposure to 0.34‰ salinity \ (x 13,000).
- 110-5 Bass pseudobranch epithelial cells (ep) after 24 hours exposure to 0.34‰ salinity. Many of the cells are disrupted and vacuolated (x 5830).



- 111-1 'Pseudobranch type' cells (pc) of trout pseudobranch 4 hours after injection of acetazolamide (l mg/kg). The mitochondria (m) are rounded up and quite dense. Electron dense bodies (db) of various sizes are seen within the cells. bc, blood channel; bm, basement membrane (x 10,400).
- 111-2 'Pseudobranch type' cells (pc) of trout pseudobranch, 24 hours after injection of acetazolamide (5 mg/kg). A large number of lipid-like droplets (d) have appeared within the cells. bc, blood channel; bm, basement membrane (x 7830).



- 112-1 Trout 'pseudobranch type' cell, 24 hours after injection of acetazolamide (5 mg/kg). The lipid-like droplets (d) are surrounded by profiles of tubular reticulum, (t) (x 31,900).
- 112-2 Trout 'pseudobranch type' cell, 24 hours after injection of acetazolamide (5 mg/kg). Some droplets (d) are associated with expanded regions of granular endoplasmic reticulum (ger) containing an amorphous material (x 28,600).
- 112-3 Trout 'pseudobranch type' cell, 24 hours after injection of acetazolamide (5 mg/kg). The cytoplasmic region of some cells is vacuolated and contains large, clear areas (c) and broken membranes. db, dense bodies; n, nucleus (x 13,000).
- 112-4 Trout 'pseudobranch type' cell (pc), 4 hours after injection of 10<sup>-3</sup>N Hcl. The cytoplasmic region of the cell is vacuolated and contains large clear areas (c) near the apical membrane. n, nucleus (x 6,855).



Trout 'pseudobranch type' cells, 4 hours after injection of 5-hydroxydopamine (50HDA).

- 113-1 The contiguous membrane between a nerve fibre and 'pseudobranch type' cell (pc) appears to have broken down (arrows). A large pale cytoplasmic region (pr) surrounded by a broken membrane (me) is present in the apical region of the 'pseudobranch type' cell. g, glycogen granules; n, nucleus; nf, nerve fibre; v, vesicles (x 17,955).
- 113-2 Apical region of the 'pseudobranch type' cell (pc) containing a large pale cytoplasmic region (pr) surrounded by membranous (me) and tubular (t) profiles. A nearby nerve fibre (nf) contains some lysosome-like dense bodies (db). i, interstitial tissue; n, nucleus (x 20,670).



Serial sections of trout pseudobranch, 4 hours after injection of 5-hydroxydopamine.

- 114-1 Large pale cytoplasmic expansions (e) appear to arise from the end of some unmyelinated nerve fibres (nf) in the terminal plexus. Small dense bodies and myelin figures (mf) may be found in these regions. m, mitochondria of nerve fibre; pc, 'pseudobranch type' cell (x 20,670).
- 114-2 Serially sectioned area of that shown in 114-1 revealing the presence of more dense bodies and myelin figures (mf) in the expanded regions (e), and the appearance of a nerve fibre containing vesicles (v) and glycogen (g). pc, 'pseudobranch type' cell; m, mitochondria of nerve fibre; nf, nerve fibre (x 20,670).



- 115-1 Large neuron of trout pseudobranch, 4 hours after injection of 5-hydroxydopamine. The mitochondria (m) are vacuolated and there is some fragmentation of the granular endoplasmic reticulum. A degenerating nerve fibre (d) is seen adjacent to the neuron. go, Golgi complex; ly, lysosome; nf, nerve fibre (x 8415).
- 115-2 Degenerating nerve fibre (d) adjacent to neuron (n) shown in 115-1. A possible synaptic region (sy) can be seen. There appears to be a breakdown of membranes between the nerve fibre and the neuron (arrows). Membranous profiles and a dense body (db) are seen within the fibre. nf, nerve fibre; m, mitochondria; r, ribosomes (x 20,280).
- 115-3 Neurons of trout primary plexus, 24 hours after injection of 5-hydroxydopamine. The mitochondria are completely vacuolated (va) and the granular endoplasmic reticulum (ger) is swollen and disorganised. Some nerve fibres (nf) are also vacuolated. nu, nucleus of neuron; sc, Schwann cell nucleus (x 3470).



Trout pseudobranch after injection of 6-hydroxydopamine (60HDA).

- 116-1 After 24 hours many terminal nerve fibres (nf) have degenerated and contain vacuolated membranous regions and dense bodies (db). Lipid-like droplets (d) are seen within the 'pseudobranch type' cells (pc) (x 19,890).
- 116-2 The apical region of some 'pseudobranch type' cells
   (pc) was vacuolated (va) 24 hours after injection.
   Broken membranes and lamellar figures (lm) were also
   seen.
   d, lipid-like droplet (x 12,750).
- 116-3 After 48 hours the number of lipid droplets had increased and in some cells they started coalescing into larger droplets (d) (x 16,380).



Multifibre recordings from trout pseudobranch nerve.

- 117-1 During perfusion of standard solution (spontaneous activity). Most activity is low level Type A and Type B activity with occasional large spikes of Type A.
- 117-2 Spontaneous activity within the first 15 minutes of perfusion with standard solution consisting mainly of irregular Type B activity.
- 117-3 Bursts of Type A activity induced by stroking the primary afferent artery with a glass rod.
- 117-4 Intense activity A caused by perfusion with 250 mMl<sup>-1</sup> NaCl solution.
- 117-5 Intense activity B during perfusion of an hypoxic solution (10 torr).
- 117-6 Intense activity B during perfusion with a hypo-osmotic solution (155 m Osml<sup>-1</sup>).

10ms

Series of successive multifibre recordings from trout pseudobranch nerve during perfusion with standard solution over a period of 20 minutes. The number of spikes per volley of type A activity gradually increased although no experimental stimulus was applied. It is possible that they are caused by brief pulses in perfusion pressure, due to a build up of vascular resistance in the preparation.

111 1111 100µV

10ms