CHAPTER-4

RESULTS
RESULTS

I. Under Normal Condition

A. Morphology of Interrenal and Chromaffin tissues

(i) *H. fossilis* and *C. batrachus*

The kidney is retroperitoneal and posterodorsal in position and lies on either side of the vertebral column separated from the body cavity by tough connective tissue septum (Fig. 7a). It consists of two distinguishable

hk = head kidney,  ab = air bladder,  tk = trunk kidney
parts - the head kidney (pronephros) and the main kidney (opisthonephros) which is the functional excretory organ in the adults. The head kidney is well separated from the main kidney by the air bladder, (Fig.7a) and the only connection between the two is the posterior cardinal vein. The head kidney lies one on either side of the vertebral column and each one is divided into two triangular lobes (Fig.7b). The bilobed head kidney comes to lie on the anterior surface of the air bladder very close to the heart.

(ii) *C. carpio* and *C. auratus*

![Image of dissected C. auratus kidney](image)

Fig. 8. Photograph of a dissected *C. auratus* showing position of kidney (k) (opisthonephros).

The kidney (opisthonephros) lies retroperitoneally and posterodorsally on either side of the vertebral column (Fig. 8). The kidneys are fan shaped in structure with a broad fan shaped anterior region and narrow posterior
region. The posterior ends are fused together along the mid line leaving the anterior broad ends free. No separate head kidney is present in *C. carpio* and *C. auratus*.

**B. Histology of the interrenal and chromaffin tissues**

**I. Light Microscopy**

(i) *H. fossilis*

Histologically, the head kidney comprises largely of haematopoietic tissue, interrenal cells, chromaffin cells (adrenal tissue), melanomacrophages cells, posterior cardinal vein and their branches.

The most anterior part of the main kidney (the opisthonephros) is also considered as the head kidney as histologically, it is composed of haematopoietic tissue, the interrenal and chromaffin cells, cardinal vein and their branches. Very few renal tubules are seen in this part of the head kidney.

**Interrenal tissue**- In the head kidney, the interrenal and chromaffin tissues do not present a definite arrangement. The interrenal tissue may be present as cluster of cells or arranged in one or several layers (2-3 in numbers) surrounding or in vicinity of the posterior cardinal vein (pcv) and its branches (Fig. 9a). The interrenal cells are either polygonal or oblong in shape and have a round nucleus with a distinct

![Fig. 9a. Section through head kidney of *H. fossilis* (control) showing interrenal (ic) and chromaffin (ch) tissues, post cardinal vein (pcv). X40](image-url)
nucleolus. In the head kidney, the interrenal tissues are largely seen in several cords closely apposed to the vein and their branches where as in the anterior kidney (opisthonephros), they occur as cords / clusters of interrenal cells scattered in the haematopoietic tissues in between the few renal tubules. The cells could be easily differentiated from the surrounding haematopoietic tissues by their staining characteristics – the interrenal cells stain light pink with distinct nucleus and nucleolus.

**Chromaffin tissues** – The chromaffin tissues consist of cells which are present singly or in groups of 2-3 cells embedded in the wall of the post cardinal vein, with or without the interrenal tissues. In the head kidney and in the anterior kidney, the chromaffin cells may also be seen interspersed with the interrenal cells among the haematopoietic tissues (Fig. 9b). The cells are round / oval shape in appearance and larger than the interrenal cells. They have a round nucleus and clear cytoplasm. The chromaffin cells can be distinguished as brown stained cells due to the fixation in Orth’s fluid. Sometimes granules may be seen in the cytoplasm of these cells. The number of interrenal cells is much more than that of chromaffin cells, whereas the number of chromaffin cells are comparatively more in the head kidney than in the anterior part of the main kidney.
Morphometric measurements - The interrenal cells are either polygonal or oblong in shape and have a round nucleus. The cells measure 4.81µ in size. In contrast, the chromaffin cells are rounded / oval shape in appearance, in size larger than the interrenal cells and measure 5.20 µ (Table-I). They have a round nucleus and clear cytoplasm.

(ii)  *C. batrachus*

The histological characteristics of the adrenal tissues are more or less similar to that of *H. fossilis.*

**Interrenal tissues**- The interrenal cells are located in one or several layers (2-3 in number) and groups surrounding the post cardinal vein or its branches and intermingled with the haematopoietic tissue (Fig. 10a). They are polygonal or oblong in shape having a round nucleus. The interrenal tissues in the head kidney and the anterior kidney are largely seen in several rows mostly apposed to the vein and their branches as well as interspersed within the haematopoietic tissues in between the few renal tubules. The interrenal cells can be distinguished as light staining cells.

**Chromaffin tissues**- Chromaffin cells constituting these tissues are predominantly seen in the head kidney in groups of 3-4, 4-5 cells near the post cardinal vein as well as embedded in the vein wall. The chromaffin cells are rounded and oval in shape and have a round nucleus and clear cytoplasm. The cell membrane is clear and sometimes granules may be
present in the cytoplasm. They are distinguishable by their brown stain (Fig. 10b). The number of interrenal cells is much more than that of chromaffin cells. While the number of chromaffin cells are more in the head kidney few chromaffin cells along with the interrenal cells are seen in the anterior kidney.

Morphometric measurements-
The interrenal cells are polygonal or oblong in shape having a round nucleus and a size of 5.0 µ. The chromaffin cells are rounded and oval in shape and have a size 4.32 µ, round nucleus and clear cytoplasm.

(iii) *C. auratus*

The head kidney of goldfish which contains the adrenal tissue is located primarily in the anterior most part of the opisthnomephros. It is made up of the interrenal and chromaffin cells, haematopoietic tissues, few renal tubules and post cardinal vein and its branches.

Interrenal tissues- The interrenal tissue of goldfish, is closely associated with the post cardinal veins or their small branches. The
interrenal cells are usually present in 3-4 rows or layers of cells along the vessels (Fig. 11a and b). Lot of haematopoietic tissue and few renal tubules are present within the head kidney (Fig. 11a). The interrenal cells are mostly oval, and columnar in shape. The nuclei are centrally located, round in shape and have prominent nucleoli.

**Chromaffin tissues** - Brown stained chromaffin cells are clearly visible in the head kidney embedded in the wall of the post cardinal vein. Groups of chromaffin cells can also be seen along with the interrenal tissue (Fig. 11b). The chromaffin cells are oval and round in shape.

**Morphometric measurements** - The interrenal cells are mostly oval, and columnar in shape having a size of 4.65 µ whereas the chromaffin cells are oval to round in shape and have a size of 5.84 µ. (Table-II)

**(iv) *C. carpio***

**Interrenal tissues** - In *C. carpio*, interrenal tissue can be located in the head kidney as several clusters of cells or layers along the post cardinal vein and their branches

Fig. 11b. Zoom view of fig. 11a showing 3-4 rows of interrenal cells (ic), chromaffin cells (ch) and renal tubules (r). X40

Fig. 12a. Interrenal (ic) and chromaffin cells (ch) of *C. carpio* (control). X40 (h= haematopoietic tissue, pcv= post cardinal vein)
(Fig. 12a and b). The interrenal cells are round, columnar and polygonal in shape.

**Chromaffin cells**- Chromaffin cells can be distinguished by their characteristically brown stain. They are clearly visible in the head kidney embedded in the walls of the cardinal vein and the smaller branches. Groups of chromaffin cells were also located in the haematopoietic tissues along with the interrenal tissues (Fig. 12b). The chromaffin cells are polygonal or oval in shape.

**Morphometric measurements**- The interrenal cells are round, columnar and polygonal in shape and have a size of 4.60 µ. The chromaffin cells are polygonal or oval in shape and have a diameter of 5.34 µ. (Table-III)

**II  Electron Microscopy**

(i)  **H. fossilis**

**Ultrastructure**

Semithin sections of *H. fossilis* show toluidine blue stained light blue interrenal cells and dark blue chromaffin cells separated by wide intercellular spaces (Fig. 13). In the head kidney, cluster of interrenal and chromaffin cells are seen in close proximity to the veins. Lots of intercellular spaces are present in between the cells. The chromaffin cells can also be located in the wall of the cardinal vein and their branches.
Fig. 13. Semithin section of the head kidney of *H. fossilis* (control) showing interrenal cells (ic), chromaffin cells (ch) with intercellular spaces (is) in between them and haemopoietic tissues (h). X40

Fig. 14a. Electron micrograph of interrenal cell of *H. fossilis* (control) showing large mitochondria (mt) with vesicular cristae, free and grouped ribosome (r), small dense bodies (db) and lysosome (l).
Fig. 14b. Electron micrograph of interrenal cell of *H. fossilis* (control) showing heterochromatic nucleus (n), mitochondria (mt) and lysosomes (l).

Fig. 14c. Electron micrograph of chromaffin cell of *H. fossilis* (control) showing noradrenaline granules (ng), nucleus (n) adjacent interrenal cell (ic) and collagen fibres (cf). (Note the presence of large intercellular spaces (is) between the cells).
Fig. 14d. Electron micrograph of the adrenal tissues of *H. fossilis* (control) showing chromaffin cells (ch) and interrenal cells (ic) in tight contact.

Fig. 14e. Electron micrograph of chromaffin cell of *H. fossilis* (control). Surrounded by collagen fibres (cf) and microfibrils (mf). Note the pleiomorphic noradrenaline granules (ng) in the chromaffin cells.
Fig. 14f. Electron micrograph of chromaffin cells of *H. fossilis* (control) showing noradrenaline chromaffin cells (nch) adjacent to interrenal cell (ic).

Fig. 14g. Electron micrograph of adrenaline chromaffin cells of *H. fossilis* (control) showing adrenaline granules (ag) and large nucleus (n).
**Interrenal cells** - The interrenal cells present an appearance typical of steroidogenic cells. The cytoplasm is rich in free and grouped ribosomes, small dense bodies, lysosomes and large number of electron lucent lipid droplets (Fig. 14a). Mitochondria are large, numerous, round or ellipsoid and have dense matrices, and tubulolamellar / vesicular cristae (Fig. 14a). Smooth endoplasmic reticulum are present in the cytoplasm, usually in the form of long cisternae and vesicles, which are evenly distributed throughout the cytoplasm. The nuclei are large, round with clumps of heterochromatin, and have prominent, eccentrically placed nucleoli (Fig. 14b and c). A large number of intercellular spaces can be seen in between the cells (Fig. 14c).

**Chromaffin cell** - The chromaffin cells are present in close contact with the interrenal cells, usually interdigitating with them (Fig. 14d). Groups of chromaffin cells are surrounded by collagen fibres and microfibrils (Fig. 14e). They are typical neuroendocrine cells with numerous pleiomorphic strongly electron dense granules (Fig. 14e and g). Nucleus is large, oval, and have light heterochromatin. The cells can be differentiated as adrenaline (Fig. 14g) and noradrenaline cells (Fig. 14f) on the basis of the electron density of the chromaffin granules. The adrenaline cells have large number of vesicles of moderate electron opacity and few granules showing aggregates of dense electron opaque material. The noradrenaline cells, which are more numerous, have a predominance of pleiomorphic, highly electron dense granules with an eccentric dense core surrounded by a clear halo (Fig. 14c). In both the types of cells, the entire cytoplasm is occupied by these granules with traces of endoplasmic reticulum and golgi bodies.
Fig. 15a. Electron micrograph of head kidney of *C. auratus* (control) showing light interrenal cells (lic) and dark interrenal cells (dic) close to post cardinal vein (pcv).

Fig. 15b. Electron micrograph of columnar light interrenal cells (lic) and dark interrenal cells (dic) of *C. auratus* (control) having large nucleus (n) with conspicuous nucleolus (nu).
Fig. 15c. Electron micrograph of interrenal cell of *C. auroa*us (control) showing numerous mitochondria (mt) having dense matrix and tubulo-vesicular cristae.

Fig. 15d. Electron micrograph of interrenal cell of *C. auroa*us (control) showing free and grouped ribosomes (r) and endoplasmic reticulum (er) in the cytoplasm.
(ii) *C. auratus*

**Ultrastructure**

**Interrenal cells** – Semithin sections show columnar and polygonal light and dark interrenal cells which appear spongy and are present closely apposed to the vein wall. Some cells are also seen in between the renal tubules.

The interrenal cells can differentiated into light and dark cells on the basis of cytoplasmic density (Fig. 15a and b). The nucleus is large with conspicuous nucleolus and has little heterochromatin in the light cells whereas in the dark cells, dense heterochromatin is found along the inner surface of the nuclear membrane. Mitochondria are numerous, having dense matrix and tubulo-vesicular cristae (Fig. 15c). In both the types of cells, a large number of smooth endoplasmic reticulum which traverse the cytoplasm as long cisternae, are predominantly seen. Both free and grouped ribosomes are abundantly present in the cytoplasm (Fig. 15d).

**Chromaffin cells** – The chromaffin cells are polygonal to columnar in shape and are characterized into two types - the adrenaline cells and the noradrenaline cells depending upon the electron density of the granules present in the cytoplasm. Strongly dense pleiomorphic granules are numerous in the noradrenaline cells whereas the adrenaline cells contained more of electron lucent granules and those with moderate opacity. The cellular densities of both the cells were equal. Both the two types of chromaffin cells contain a distinct nucleus which is relatively large occupying a major part of the cell and with a prominent nucleolus.
2. Experimental Results: Effect of Hypoxia

Morphological changes were observed in the interrenal and chromaffin cells of *H. fossilis*, *C. auratus* and *C. carpio*, after exposure to 5 hr and 12 hr duration in each of moderate and acute hypoxic conditions. Since only *H. fossilis* and *C. auratus* were examined ultrastructurally, for these fishes alone, the changes at the ultrastructural levels are reported here.

A. Exposure to Moderate Hypoxia – (30-50% oxygen saturation)

(i) *H. fossilis*

Morphometric and histological changes

Exposure to 5 hr duration
No changes were observed in the interrenal cells and chromaffin cells as compared to that of the control cells (Fig. 16a and b). (Table-I)

Exposure to 12 hr duration

The size of interrenal cells increased as compared to that of control (Table-I). After exposure to 12 hr of moderate hypoxia, the interrenal cells measured 7.3µ in diameter, and appeared as large polygonal cells with dense nuclei. The bands/cords of interrenal cells increased in thickness as compared to that seen in control and after 5 hr of moderate hypoxia (Fig. 16c and d). No change was observed in the size and shape of chromaffin cells. (Table-I).
Fig. 16a. Interrenal (ic) and chromaffin cells (ch) of *H. fossilis* under 5 hr moderate hypoxia. No changes were observed in the interrenal and chromaffin cells. X40

Fig. 16b. Interrenal (ic) and chromaffin (ch) cells distributed among the haematopoietic tissues (h) in *H. fossilis* after exposure to 5 hr moderate hypoxia. X40

Fig. 16c. Several bands of interrenal cells (ic) seen in *H. fossilis* under exposure to 12 hr moderate hypoxia. Note the densely stained nuclei. X40
(ch= chromaffin cell, h= haematopoietic tissue)

Fig. 16d. Thick bands of interrenal cells (ic) seen in *H. fossilis* after exposure to 12 hr moderate hypoxia. X40
(ch= chromaffin cell, h= haematopoietic tissue)
<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Normoxia (Control)</th>
<th>5 hr Moderate Hypoxia</th>
<th>12 hr Moderate Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved O₂ Levels (% Oxygen Saturation)</td>
<td>80-90%</td>
<td>30-50%</td>
<td>30-50%</td>
</tr>
<tr>
<td>Size of Chromaffin Cells (Mean Value, µm)</td>
<td>5.20 ± 0.388</td>
<td>5.52 ± 0.300</td>
<td>5.82 ± 0.572</td>
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<tr>
<td>Size of Interrenal Cells (Mean Value, µm)</td>
<td>4.81 ± 0.098</td>
<td>6.02 ± 0.636</td>
<td>7.35 ± 0.387</td>
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<tr>
<td>% Increase In Interrenal Cell Size</td>
<td>–</td>
<td>25.15</td>
<td>52.80</td>
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</tbody>
</table>

Table- 1  Mean values of chromaffin and interrenal cell sizes in *H. fossilis* following 5 hr and 12 hr exposure to moderate hypoxia.

**Ultrastructural changes**

After 12 hr exposure of moderate hypoxia large interrenal cells were seen in semithin section (Fig. 17). Ultrastructurally the only change seen in the interrenal cells is the clumping of heterochromatin in the nuclei (Fig. 18a). Small perinuclear spaces can also be seen (Fig. 18b). The mitochondria show less dense matrix, and long cisternae of smooth endoplasmic reticulum traverse the cytoplasm of the cell.
Fig. 17. Large interrenal cells (ic) with conspicuous nuclei and chromaffin cells seen in semithin section of *H. fossilis* under exposure to 12hr moderate hypoxia. X40

Fig. 18a. Electron micrograph of enlarged interrenal cells of *H. fossilis* after exposure to 12hr moderate hypoxia showing clumping of heterochromatin in the nuclei.

Fig. 18b. Electron micrograph of enlarged interrenal cells of *H. fossilis* after exposure to 12hr moderate hypoxia. Showing long cisternae of smooth endoplasmic reticulum (sER), and heterochrometic nuclei (n).
(ii)  *C. auratus*

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Normoxia (Control)</th>
<th>5 hr Moderate Hypoxia</th>
<th>12 hr Moderate Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved O$_2$ Levels (% Oxygen Saturation)</td>
<td>80-90%</td>
<td>30-50%</td>
<td>30-50%</td>
</tr>
<tr>
<td>Size of Chromaffin Cells (Mean Value, µm)</td>
<td>5.84 ± 0.300</td>
<td>5.80 ± 0.318</td>
<td>5.68 ± 0.429</td>
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<tr>
<td>Size of Interrenal Cells (Mean Value, µm)</td>
<td>4.65 ± 0.136$^a$</td>
<td>5.44 ± 0.176$^a$</td>
<td>8.45 ± 0.288$^b$</td>
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<td>% Increase In Interrenal Cell Size</td>
<td>–</td>
<td>16.98</td>
<td>81.72</td>
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</table>

Table- II  Mean values of chromaffin and interrenal cell size in *C. auratus* after exposure to 5 hr and 12 hr to moderate hypoxia. Different lower case letters indicate significant differences (p<0.05).

**Morphometric and histological changes**

The following morphological changes were observed in the interrenal and chromaffin cells of *C. auratus* after exposure to different levels of moderate hypoxia for different durations of time. (Table-II)
Exposure to 5 hr duration

Exposure to 5 hr of moderate hypoxia (40% oxygen saturation) produced very small change in the interrenal cells of *C. auratus* as compared to control. The interrenal cells show a small increase in size 5.80µ in

![Image](image1.png)

Fig. 19a. Very small increase in size of interrenal cells (ic) of *C. auratus* exposed to 5 hr moderate hypoxia. X40 (ch= chromaffin cell, h= haematopoietic tissue)

![Image](image2.png)

Fig. 19b. Zoom view of fig. 19a. Distinct nuclei can be clearly seen. X40

*C. auratus*. (Table-II). The cells can be easily differentiated and show no change in the staining characteristics (Fig. 19a and b).

No change was observed in the size of chromaffin cells. (Table-II).
**Exposure to 12 hr duration**

Several thickened bands of interrenal tissues were observed among the haematopoietic tissue (Fig. 19c and d). The diameter of the interrenal cells showed a significant (p<0.05) increase as compared to the control: the cells measure 8.45µ in *C. auratus* (Table-II). The nuclei stained dense and only a narrow rim of clear cytoplasmic area was visible.
**Ultrastructural changes**

After exposure to 12 hr of moderate hypoxia, several changes were observed at the ultrastructural level. In the dark interrenal cells, the mitochondria have less dense matrix (Fig. 20a). In the light cells numerous small sized mitochondria and a lighter cytoplasm were observed (Fig. 20b). Abundant smooth endoplasmic reticulum (sER) were observed and the cisternae were seen traversing the cytoplasm and the cisternae either very close or encircle the mitochondria. Some of them extended as long channel opening at the cell surface (Fig. 20c and d).

**(iii) C. carpio**

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Normoxia (Control)</th>
<th>5 hr Moderate Hypoxia</th>
<th>12 hr Moderate Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved O₂ Levels (% Oxygen Saturation)</td>
<td>80-90%</td>
<td>30-50%</td>
<td>30-50%</td>
</tr>
<tr>
<td>Size of Chromaffin Cells (Mean Value, µm)</td>
<td>5.34 ± 0.132</td>
<td>5.22 ± 0.064</td>
<td>5.95 ± 0.385</td>
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<tr>
<td>Size of Interrenal Cells (Mean Value, µm)</td>
<td>4.60 ± 0.040</td>
<td>5.41 ± 0.273</td>
<td>7.79 ± 0.288</td>
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<td>% Increase In Interrenal Cell Size</td>
<td>_</td>
<td>17.60</td>
<td>69.34</td>
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Table- III  Mean values of chromaffin and interrenal cell size in *C. carpio* after exposure to 5 hr and 12 hr of moderate hypoxia.
Fig. 20a. Electron micrograph of hypertrophied interrenal cells of *C. auratus* following exposure to 12hr moderate hypoxia showing the dark interrenal cells (dic) and the mitochondria (mt) both having light cellular matrix.

Fig. 20b. Electron micrograph of enlarged interrenal cells of *C. auratus* after exposure to 12hr moderate hypoxia. Numerous mitochondria (mt) and lighter cytoplasm (ct) can be seen in the light interrenal cells.
Fig. 20c. Electron micrograph of enlarged interrenal cells of *C. auratus* after exposure to 12 hr moderate hypoxia showing long cisternae of smooth endoplasmic reticulum (sER) running close to and encircling the mitochondria (mt).

Fig. 20d. Electron micrograph of enlarged interrenal cells of *C. auratus* after exposure to 12 hr moderate hypoxia. Smooth endoplasmic reticulum (sER) were seen traversing the cytoplasm and opening at the cell surface and mitochondria seen with light matrix.
Morphometric and histological changes

Exposure to 5 hr duration

Exposure to 5 hr duration of moderate hypoxia, did not produce any noticeable change in either the interrenal or chromaffin cells of the common carp (Fig. 21a). (Table-III)

Exposure to 12 hr duration

After 12hr exposure to moderate hypoxia conditions, small changes in the size of interrenal cells were observed. (Fig. 21b and c, Table-III ). The interrenal cells measured $7.79\mu$ in diameter, the cytoplasm stained light and prominent nucleoli were visible.
B. Exposure to Acute Hypoxia – (<30% oxygen saturation)

(i) *H. fossilis*

**Morphometric and histological changes**

Following morphological changes were observed after 5 hr. and 12 hr. exposure.

**Exposure to 5 hr duration**

Increased thickness of bands of interrenal cells were seen in the area around the post cardinal vein and their branches (Fig. 22a). The interrenal cells measured 8.63µ which is significantly higher (p<0.05) as compared to that of control cells and there was 79% increase in cell size. The cell boundaries of the interrenal cells were not clearly visible and the nuclei also stained light. The cells present a spongy appearance (Fig. 22b).

![Fig. 22a](image1.png) Significant (P<0.05) increase in interrenal cells of *H. fossilis* under exposure to 5 hr acute hypoxia. Cell boundaries not clearly seen. X40  

![Fig. 22b](image2.png) Zoom view of fig. 22a. Show the interrenal and chromaffian cells were seen in the around PCV vein and their branches. X40
No change was seen in the chromaffin cell size and number. (Table-IV). Large number of red blood cells were seen in the haematopoietic tissues.

**Exposure 12 hr duration**

Further increase in the size of the interrenal cells was observed as compared to that of control, moderate hypoxia and 5 hr acute hypoxia. The interrenal cells measured 10.66 µ which is significantly higher (p<0.05) as compared to control. The thickness of interrenal bands was also considerably increased as compared to control, moderate and 5 hr acute hypoxia, and they are visible as several spongy and frothy bands occupying the haematopoietic tissue (Fig. 22c). The nucleus stains light and the cell membranes were not clearly seen (Fig. 22d).

No change in chromaffin cells were observed (Table-IV). Large number of blood cells can be seen occupying the haematopoietic tissues.
<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Normoxia (Control)</th>
<th>5 hr Acute Hypoxia</th>
<th>12 hr Acute Hypoxia</th>
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<tbody>
<tr>
<td>Dissolved O$_2$ Levels (% Oxygen Saturation)</td>
<td>80-90%</td>
<td>&lt; 30 %</td>
<td>&lt; 30 %</td>
</tr>
<tr>
<td>Size of Chromaffin Cells (Mean Value, µm)</td>
<td>5.20 ± 0.388</td>
<td>6.05 ± 0.302</td>
<td>6.09 ± 0.564</td>
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<tr>
<td>Size of Interrenal Cells (Mean Value, µm)</td>
<td>4.81 ± 0.098$^a$</td>
<td>8.63 ± 0.213$^b$</td>
<td>10.66 ± 0.930$^b$</td>
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<tr>
<td>% Increase In Interrenal Cell Size</td>
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<td>79.41</td>
<td>121.62</td>
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</tbody>
</table>

Table-IV  Mean values of chromaffin and interrenal cell sizes in *H. fossilis* exposed to 5 hr and 12 hr acute hypoxia. Different lower case letters in rows indicate significant differences.

**Ultrastructural changes**

Ultrastructurally, several changes were seen in the interrenal cells and chromaffin cells of *H. fossilis* after exposure to 12 hr of acute hypoxia.

**Interrenal cells**

In the interrenal cells, the mitochondria appear smaller in size, numerous and are more elongated and dumbbell-shaped (Fig. 23a). A number of vesicles of smooth endoplasmic reticulum are seen in the cytoplasm (Fig. 23b). The plasma membrane appears irregular and frayed. The nuclei are euchromatic with wide perinuclear spaces and the nucleoli appear smaller in size (Fig. 23a). The number of electron lucent lipid droplets seen in the control cells is drastically reduced, and the
Fig. 23a. Electron micrograph of hypertrophied cells of *H. fossilis* after exposure to 12hr acute hypoxia showing numerous, smaller, elongated to dumbbell-shaped mitochondria (mt). Note the wide perinuclear gap (→).

Fig. 23b. Electron micrograph of interrenal cell (ic) and chromaffin cell of *H. fossilis* after exposure to 12hr acute hypoxia showing dumbbell shaped mitochondria (mt) and vesiculated smooth endoplasmic reticulum (sER) in the cytoplasm. Chromaffin cells (ch) are also seen.
Fig. 23c. Electron micrograph of noradrenaline chromaffin cell (nch) and interrenal cell (ic) of *H. fossilis* after exposure to 12hr acute hypoxia. The nucleus (n) is euchromatic with a prominent nucleolus. Note vesiculated endoplasmic reticulum (ER) in both the cells.

Fig. 23d. Electron micrograph of noradrenaline chromaffin cell (nch) of *H. fossilis* after exposure to 12hr acute hypoxia showing reduced halo around the dense granules and increase in the number of clear vesicles.
intercellular spaces appear filled with electron lucent material (Fig. 23a).

**Chromaffin cell**

The chromaffin cells also present an altered picture in the noradrenaline cells (Fig. 23c and d). Numerous dense granules are seen but they do not appear to be surrounded by the clear wide halo as seen in control cells. The number of eccentric dense granules are greatly reduced in number. Numerous granules of variable densities are predominantly seen. Very few and smaller electron lucent granules are seen as compared to control cells. The nucleus is euchromatic with a prominent nucleolus.

(ii) **C. auratus**

**Morphometric and histological changes**

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Normoxia (Control)</th>
<th>5 hr Acute Hypoxia</th>
<th>12 hr Acute Hypoxia</th>
</tr>
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<tbody>
<tr>
<td>Dissolved O(_2) Levels (% Oxygen Saturation)</td>
<td>80-90%</td>
<td>&lt; 30 %</td>
<td>&lt; 30 %</td>
</tr>
<tr>
<td>Size of Chromaffin Cells (Mean Value, (\mu)m)</td>
<td>5.84 ± 0.300</td>
<td>5.74 ± 0.461</td>
<td>5.64 ± 0.204</td>
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<tr>
<td>Size of Interrenal Cells (Mean Value, (\mu)m)</td>
<td>4.65 ± 0.136(^a)</td>
<td>10.11 ± 0.115(^b)</td>
<td>11.00 ± 0.025(^b)</td>
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<td>% Increase In Interrenal Cell Size</td>
<td>_</td>
<td>117.41</td>
<td>136.55</td>
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Table- V    Mean values of chromaffin and interrenal cell size in C. auratus under exposure to 5 hr and 12 hr acute hypoxia. Different lower case letters in rows indicate significant differences.
Exposure to 5 hr duration

In *C. auratus* the exposure to 5 hr duration of acute hypoxia produced thickened bands of interrenal tissues seen scattered in the haematopoietic tissues as compared to that in moderate hypoxia (Fig. 24a). The diameter of interrenal cells measure 10.11µ in *C. auratus* which is a significant (p<0.05) increase as compared to the control. The nuclei appeared in distinct as irregular clumps and cell boundaries were not clearly discernible. The interrenal cells present a spongy appearance (Fig. 24b).

No change was observed in the size of chromaffin cells. (Table-V)

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Fig. 24a. Significant (p<0.05) increase in interrenal cell size of *C. auratus* after exposure to 5 hr acute hypoxia. Several thickened bands of interrenal tissues (ic), seen surrounded by haematopoietic tissue (h). X40 (ch=chromaffin cells)

Fig. 24b. After exposure to 5 hr acute hypoxia. Note the enlarged interrenal cells (ic). X40 (pcv=post cardinal vein, ch=chromaffin cell)
Exposure to 12 hr duration

The interrenal tissues present a spongy and frothy appearance after exposure to 12 hr of acute hypoxia (Fig. 24c). Cell size of the interrenal cells showed a significantly (p<0.05) higher value of 11.00µ in *C. auratus* (Table-V) as compared to the control and thick bands of the interrenal tissues were seen (Fig. 24c). Large number of blood cells were seen in the anterior kidney region in *C. auratus*.

Ultrastructural changes

In semithin sections (Fig. 25a), the interrenal cells are considerably enlarged and have lot of intercellular spaces in between them. Nuclei are not visible and the cells appear vacuolated. Dark cells also appear enlarged and show denser nuclei.

In the light interrenal cells, smaller and numerous mitochondria, having less dense matrix were observed. They appeared elongated or dumbbell-shaped. In the cytoplasm small broken strands of cisternae were visible in the form of spherical vesicles (Fig. 25b and c). Several large spaces having electron lucent material were also observed (Fig. 25d). The outer plasma membrane appeared irregular in outline, and highly frayed with several dense vesicles close to the periphery (Fig. 25e). Nucleus was euchromatic. In the dark cells, clumping of nuclear heterochromatin with
Fig. 25a. Semithin section of *C. auratus* following exposure to 12hr acute hypoxia. Note the enlarged, vacuolated interrenal cells (ic) and having lot of intercellular spaces (is) in between them post cardinal vein (pcv).

Fig. 25b. Electron micrograph of interrenal cell of *C. auratus* after exposure to 12hr acute hypoxia showing nuclear changes in nucleus (n) of dark cells (dic) and vacuolated light cell (lic).

Fig. 25c. Electron micrograph of degenerating light interrenal cell of *C. auratus* following exposure to 12hr acute hypoxia showing elongated and dumbbell shaped mitochondria (mt), vesiculated endoplasmic reticulum (ER) and vacuolated cytoplasm (c).
Fig. 25d. Electron micrograph of dark interrenal cell of *C. auratus* after exposure to 12hr acute hypoxia showing prominent nucleoli (nu), smaller and dividing mitochondria (mt) and lighter cell matrix.

Fig. 25e. Electron micrograph of light interrenal cell of *C. auratus* after exposure to 12hr acute hypoxia showing irregular and frayed peripheral cell membranes (cm) and small dense vesicles (v) close to outer cell membrane.
prominent nucleoli and lighter cellular matrix was observed. Few strands of smooth endoplasmic reticulum were observed (Fig. 25c and d).

(iii) *C. carpio*

**Morphometric and histological changes**

**Exposure to 5 hr duration**

Exposure to 5 hr of acute hypoxia, produced thick bands of interrenal cells visible in the haematopoietic tissues, in between the renal tubules and around the cardinal veins (Fig. 26a). The interrenal cells showed a significant increase in size (p < 0.05) with the cells measuring 9.41µ diameter (Table-VI). The cells appeared spongy and the nuclei showed irregular clumping.

![Fig. 26a. Significant (p<0.05) increase in interrenal cells (ic) of *C. carpio* after exposure to 5 hr acute hypoxia. Cell boundaries not clearly seen. X40](image1)

![Fig. 26b. After exposure to 12 hr acute hypoxia, interrenal cells (ic) present a spongy and frothy appearance cells distinguishable by their light staining nuclei. X40 (ch=chromaffin cells)](image2)
<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Normoxia (Control)</th>
<th>5 hr Acute Hypoxia</th>
<th>12 hr Acute Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved O$_2$ Levels (% Oxygen Saturation)</td>
<td>80-90%</td>
<td>&lt; 30%</td>
<td>&lt; 30%</td>
</tr>
<tr>
<td>Size of Chromaffin Cells (Mean Value, µm)</td>
<td>5.34 ± 0.132</td>
<td>5.73 ± 0.251</td>
<td>6.01 ± 0.275</td>
</tr>
<tr>
<td>Size of Interrenal Cells (Mean Value, µm)</td>
<td>4.60 ± 0.040$^a$</td>
<td>9.41 ± 0.735$^b$</td>
<td>10.83 ± 0.877$^b$</td>
</tr>
<tr>
<td>% Increase In Interrenal Cell Size</td>
<td>–</td>
<td>104.56</td>
<td>135.43</td>
</tr>
</tbody>
</table>

Table- VI  Mean values of chromaffin and interrenal cell size in *C. carpio* after exposure to 5 hr and 12 hr acute hypoxia. Lower case letters in rows indicate significant differences.

**Exposure to 12 hr duration**

The interrenal tissues present a spongy and frothy appearance after exposure to 12 hr of acute hypoxia (Fig. 26b and c). Cell size of the interrenal cells showed a significantly (p<0.05) higher value of 10.83µ in *C. carpio* (Fig. 26c, Table-VI) as compared to the control and thick bands of the interrenal tissues were seen. Large number of blood cells were seen in the anterior kidney region in *C. carpio*.

Fig. 26c. Thick bands of interrenal cells (ic) and chromaffin cells (ch) of *C. carpio* after exposure to 12 hr acute hypoxia. X40

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