HISTOLOGY AND CYTOLOGY OF THE LIVER OF
ICHTHYOPHIS GLUTINOSUS (LINN.) AND
URAEOTYPHUS NARAYANI
(SESCHACHAR)—PART I

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Introduction

A study of the literature on the structure of the liver reveals that our
knowledge of the histology and cytology of the liver of Gymnophiona is
singularly defective. Weidersheim was the first to give an account of the
main anatomical features of Cæcilia (Ichthyophis) rostrata. The Sarasins in
their monograph on the development and anatomy of Ichthyophis glutin-
osus, have briefly referred to the general disposition of the liver. Recently
Chatterjee has given an account of the gross anatomy of Uraeotyphlus menoni.
In the present communication an attempt is made to describe the minute
structure of the liver of two forms of Gymnophiona, Ichthyophis glutinosus
and Uraeotyphlus narayani, with special reference to the cytoplasmic
inclusions.

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guidance the work was carried out. I am thankful to him for his kind and
helpful advice and constant encouragement. My grateful thanks are also
due to Dr. B. R. Seshachar for much assistance.

Material and Methods

The specimens of Ichthyophis glutinosus were obtained from the malnad
parts of Mysore (Kotigehar, Kadur District), and live specimens of
Uraeotyphlus narayani were purchased from the Biological supplies, Kottayam
(Travancore). Mature specimens measuring from six to nine inches have
been used. The live specimens were fixed on to the dissecting board and
the abdomen opened. The liver was quickly removed and placed in a small
glass dish, and then rapidly cut into small pieces as required and fixed.
The following fixatives were employed: (1) Bouin's fluid, (2) Regaud's
Formol Bichromate, (3) Champy-kull. (4) Da-Fano's fixative with cobalt

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nitrate, (5) Mann-Kopsch and (6) Kolatchew’s method as modified by Nassonow.

Bouin’s fluid proved the best for general histological studies; the second and third were generally employed for the study of mitochondria. In these cases sections were stained with iron hæmatoxylin. The last three methods were used with satisfactory results for the investigation of both mitochondria and Golgi bodies. Paraffin sections of varying thickness (8μ to 10μ) were cut, and sections 3μ, 4μ and 6μ thick proved useful for the study of the cytoplasmic inclusions.

Observations: A. General Disposition of the Liver

Of the glands associated with the digestive tract, the liver is by far the largest (Fig. 1. Lr.). In both Ichthyophis glutinosus and Uraeotyphlus narayani, the liver is an elongated and lobulated organ compressed dorsoventrally as in Uraeotyphlus menoni described by Chatterjee. It is grey-green in colour in the well-fed condition. In length it is almost equal to one-third the total length of the body. In Uraeotyphlus narayani as in Uraeotyphlus menoni, the liver is situated in the middle third region of the body, and measures about 3.1 inches in length, and in Ichthyophis glutinosus it is slightly anterior in position and measures about 3 inches in length. In both the forms the liver is situated posterior to the heart. In these species the medio-lateral edge of the liver is applied to the wall of the stomach while the free edge is divided into a variable number of segments by transverse grooves. Chatterjee’s terms “lobes” for the segments and “circular grooves” do not appear to me to be correct.

In the liver of Uraeotyphulus narayani, as many as forty segments, and in that of Ichthyophis glutinosus thirty-eight segments have been noticed. The segments in the anterior and posterior regions of the liver more or less overlap one another and are smaller than those in the middle region. It may be stated that in the liver of younger specimens the segments are uniformly small, and that the overlapping is less marked.

The liver in these two species of Gymnophiona when compared with that of an Anuran, is relatively simple being made up of only one lobe. The elongation of the organ in these forms may be correlated with the great elongation of the body.

The Gall-Bladder, a spherical greenish organ situated postero-ventrally, not far from the posterior end of the liver, is attached to the liver by connective tissue and peritoneum. When full it has a smooth outer surface. Histologically it resembles the gall-bladder of Anura, excepting that the folds of the
mucosa layer are not so prominent as in Anura (Fig. 15, a and b). The
cystic duct (C.D.) which is small in both the species has a simple course,
and it unites with the main hepatic duct (M. H. D.) to form the ductus
choledochus (D.C.). This opens into the duodenum (D).

B. General Histology

A microscopic examination of the transverse section of the liver
shows that externally it is covered by an extremely thin delicate
peritoneal covering (Tunica Serosa) (Figs. 3, 14, per. L.). This consists of flattened cells with granular protoplasm and compressed nuclei (Figs. 3, 5, 14, C. per. L.). Immediately beneath this is a delicate connective tissue layer containing elastic fibers, which envelops the liver and forms its capsule (Figs. 3, 5, 14, C. T. L.). Next to the connective tissue layer comes the glandular tissue of the liver, which is divisible into two regions a feature not observable in the liver of Anura (Figs. 2, 3, 14, Cor. Med.). The peripheral cortical region is made up of two to three layers of closely packed, relatively small, spherical or polygonal cells with feebly granular protoplasm containing polymorphic nuclei. The occurrence of polymorphic nucleus in the cortical cell is a noteworthy feature. Wilson speaks of such nuclei as an indication of the pathogenic condition of the cells. Seshachar observes cells with polymorphic nuclei in the primary spermatagonia of Ichthyophis glutinosus, and is of opinion that these denote "a condition of great nutritive activity". It may also be possible that the polymorphic condition of the nucleus indicates an early stage in the amitotic

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Fig. 3. Enlarged view of a part of the liver to show the distinction between Cortex and Medulla. \( \times 1800 \). Fig. 4. A Medullary Cell, from Fig. 2, with the central spherical nucleus and three nucleoli. \( \times 5400 \). Fig. 5. A Cortical cell, from Fig. 2, showing the polymorphic nucleus, a Peritoneal cell (C. per. L.) and connective tissue layer (C. T. L.). \( \times 5400 \).
division of the cells. But the fact that they are found in almost all the
cells of the cortex suggests that they are not connected with the amitotic
division of the cells. Moreover it is observed that frequently the division of
the cells in the cortex is by mitosis (Fig. 7). Careful examination of the
slides failed to reveal any fact suggestive of a pathological condition.
The polymorphic nuclear condition of the cortical cells of the liver appears
to be peculiar to Apoda.

Next to the cortex is the deeper part of the liver, the medulla, consisting
of hepatic tubules (H.T., Figs. 2, 6.), hepatic sinusoids (H.S., Figs. 2, 3, 6),
bile canaliculi (b.can., Figs. 11b, 12b) and bile canals (B. can., Figs. 2, 3,
6, 7, 8).

**Hepatic Tubules.**—The structure of the deeper portion (medulla) of the
liver is, as in other Amphibia, typically tubular. The hepatic tubules enclose
a lumen or bile canal (Figs. 2, 6, 12a). The tubules appear to be closely
packed and in contact with each other except where they are separated by
hepatic sinusoids. The wall of these tubules consists of both polyhedral and
pyramidal cells, but the former type occurs in preponderance. The cell walls
are usually distinct, (Figs. 2, 4, 6, 8). It is to be noted as shown in Fig 6, that on the thin endothelial wall of the hepatic sinusoids rest the polyhedral hepatic cells.

Fig. 7. Transverse section of the liver of *Ureotyphlus* showing mitotic figures in the Cortex Bouin. × 3600.

The hepatic cells possess frequently one or rarely two spherical nuclei embedded in the cytoplasm (Figs. 4, 8.). The nuclei are usually central in polyhedral cells and are slightly excentric in pyramidal ones (Fig. 8). Frequently they contain more than one distinct nucleolus.

The cytoplasm of the hepatic cells is finely reticular, the meshes being filled with coarse granules of irregular size. These cells contain the usual cytoplasmic inclusions like the Golgi apparatus and mitochondria, and in active cells the secretory mass (S) in the *secretogenous zone*. (Sec. Z. Fig. 13).

The hepatic sinusoids constitute an important feature of the medulla. The term "hepatic sinusoid" was first employed by Minot (1900) "to indicate endothelial tubes which are wider than most capillaries, yet do not possess the coats distinctive of most arterioles and venules". These are the finer branches of the hepatic veins and arteries, and are variable in size and shape. They appear in sections as circular (Figs. 2, 3, H.S.) or elongated (Figs. 2, 7), or irregular spaces (Figs. 6, 8) between hepatic tubules. A few hepatic sinusoids occur between the cortex and medulla but none in the cortex.
Fig. 8. Transverse section of the liver (Uraotyphius) showing a medullary cell in the Metaphase stage; not all the chromosomes are shown. The pyramidal hepatic cells on the left side. Bouin. × 5400. Fig. 9. A portal canal showing the portal vein (P.V.) large hepatic ducts (H.D.), small hepatic ducts (h.d.), Hepatic arteries (H.art.) and connective tissue (C.T.) Ichthyophis. Bouin. × 1600.

The delicate endothelium of the hepatic sinusoids consists of two distinct types of cells (Fig. 6, Endo., K.C.). The first type consists of small flattened endothelial cells (Endo) with feebly granular protoplasm and small oval nuclei. Under high magnification the nucleus presents a delicate nuclear membrane enclosing a reticulum and a small nucleolus. The second type comprises the large irregularly shaped cell, the Kupffer cell [Stellate cell (K.C.)] with many cytoplasmic processes and a large spherical nucleus. The protoplasm is distinctly granular. The cytoplasmic processes extend into the hepatic sinusoids. They are known to be (1) phagocytic in function, and to be (2) active in fat metabolism and in the formation of the bile pigment [Bailey¹ and Cowdry⁴].

Much controversy exists among histologists as regards the nature of Kupffer cells. Cowdry⁴ considers them to be an integral part of the endothelium of the hepatic sinusoids. Mann¹⁰ is of opinion that they are the very much modified hepatic cells which have taken up a phagocytic function. In view of the close histological relationship existing between these cells and the
endothelium of the hepatic sinusoids, I am inclined to agree with Cowdry and consider that Kupffer cells form an integral part of the endothelium of the hepatic sinusoids and are the very much modified endothelial cells.

That the division of hepatic cells is ordinarily by amitosis has been established by histologists. Subramaniam\(^{18}\) observes "In text-books of Histology it is mentioned that the mitotic figures are not common in the normal liver cells, and my experience in the liver cells of *Rhacophorus* confirms this observation. Division of cells is by amitosis and even this was rare in the material." In the Apodan forms under investigation the occurrence of mitotic activity is noticed not only in the cortex but also in the medulla (Figs. 7, 8). While it is not possible to affirm that the hepatic cells divide mitotically on the basis of cells exhibiting mitosis, I believe that their occurrence in both cortex and medulla is of interest. It suggests that both the methods of cell division may occur in the liver of Apoda under investigation.

The *bile canaliculi*, the *bile canals* and the hepatic ducts form the duct system of the liver. This system may be considered under two sections, (1) the *intra-hepatic* system and (2) the *extra-hepatic* system. The *bile canaliculi* (b. can., Figs. 3, 11 b, 12 b), the bile canals (B. can., Figs. 2, 3, 6) and the small and large hepatic ducts (h.d., H.D.) found in the *portal canal* (Fig. 9) constitute the *intra-hepatic* system. This system commences as extremely fine canals (*bile canaliculi*) (Collateral canals of Mc Indoe\(^{11}\)) between liver cells, which empty themselves into the *bile canal* situated in the centre of the tubule.

It should here be stated that such inter-cellular *bile canaliculi* and *bile canals* are separated from the hepatic sinusoids (wherever they occur) by the thickness of one liver cell only. The *bile canaliculi* open into the *bile canals* and the latter by the widening of the lumen become converted into small *hepatic ducts*. The cells forming the wall of these *hepatic ducts* are small and cubical with spherical nuclei. These small *hepatic ducts* open into the large *hepatic ducts*, the lining cells of which are slightly columnar.

The *extra-hepatic system* comprises the *main hepatic duct* (M.H.D.), the *cystic ducts* (C.D.) and the *ductus choledochus* (D.C., Fig 1). The large and small *hepatic ducts* communicate ultimately with the main *hepatic duct* uniting with the *cystic duct* to form the *ductus choledochus* which opens into the duodenum. The function of both the systems is, as is well known, to lead the bile to the duodenum.

C. The Cytoplasmic Inclusions

1. *Mitochondria.*—There are three types of mitochondria in the liver cells (medullary cells) of *Ichthyophis* and *Uraeotyphlus*. These are (1)
granular (M), (2) rod-like (M₁) which are short and thick, and (3) filamentous (M₂), which are longer and thinner than M₁. Of these the first and the third types of mitochondria are more commonly met with.

Figs. 10a and 10b. Longitudinal section of the liver (Ichthyophis). Granular mitochondria (M) are scattered throughout the cells forming a “mitochondrial film”. Regaud. × 900.

Figs. 11a and 11b. Longitudinal section of the liver (Ichthyophis). All the three types of mitochondria (Granular M, rod-like M₁ and filamentous M₂) are found in the secretogenous zone sec. Z.), some granular mitochondria have formed a Mitochondria cap (M.C.) to the nucleus, Regaud. × 900.

Figs. 10a, 10b, 11a, 11b represent the appearance of hepatic cells in the inactive and active conditions. They show two different regions of the same section suggesting the variability in the activity of the liver cells in the different regions of the liver. The cytoplasm of the cells shown in Figs. 10a, 10b has granular mitochondria irregularly distributed in the cytoplasm. The secretory droplets are absent. These cells may therefore be considered to be inactive. In cells depicted in Figs. 11a, 11b both the rod-shaped and filamentous mitochondria occur. The granular mitochondria form a mitochondrial cap (M.C.) on that part of the nucleus facing the bile canal, whilst the other two types of mitochondria are found in the Secretogenous Zone (Sec. Z.). The cytoplasm (S′) of this zone lying between the nucleus and the bile canal is deeply stained. The
occurrence of the three types of mitochondria in the secretogenous zone it may be presumed, indicates the beginning of the active condition of the cells. Further the intimate association of mitochondria with the secretogenous zone suggests the possibility of mitochondrial influence in initiating the secretory process.

From the foregoing it is obvious that granular mitochondria are found irregularly scattered throughout the cell in the resting condition and form a "mitochondrial film". In the beginning of the secretory activity, the granular mitochondria form a mitochondrial cap to the nucleus and the rod-shaped and filamentous mitochondria are found in the secretogenous zone. The present observations on the liver cells of Apoda confirm those of Meersmann who finds filamentous mitochondria in the active and granular mitochondria in the resting hepatic cells of guinea-pig.

2. Golgi Apparatus.—The Golgi apparatus in the liver cells (medullary cells) of the forms under investigation is recognisable in three different forms—small discrete bodies (G), rods (G₁) and net-works (G.N.W.) (Figs. 12 a, 12 b, 13).

Fig. 12 a. Transverse section of a hepatic tubule (Uraeotyphlus). The golgi apparatus is simple, juxta-nuclear, discrete (G) and rod-shaped (G₁). In the right upper cell small lateral processes (L.P.) and in the left upper cell Golgi blebs are seen. There are no secretory droplets and the cells represent a phase before the beginning of the secretory activity. Da Fano. × 900. Fig. 12 b. Transverse section of the liver of Uraeotyphlus. The Golgi apparatus is becoming network-like, Golgi crescents (G₂) and some Golgi canals (G₃) are noticeable. The secretory droplets (S) are being formed in relation with the Golgi apparatus. Along the Golgi strands certain swellings, the Golgi blebs (G.B.) are differentiated. The Golgi vesicles (G.V.) are few. Mann Kopsch. × 900.

Fig. 12 a represents the transverse section of a hepatic tubule, and the cytoplasm of the cells contains the centrally placed nucleus. The Golgi bodies are discrete, and rod shaped; some of these possess lateral processes (L.P.). The apparatus occurs near the nucleus in the secretogenous
zone. This stage may be presumed to represent a phase before the beginning of the secretory activity of the hepatic cells as the secretory products are not yet visible.

Fig. 13. Transverse section of the liver of Ichthyophis. The Golgi net-work (G.N.W.), with Golgi vesicles (G.V.) is well-developed and extends throughout the secretogenous zone (Sec. Z.) which is full of secretory products (S). The Golgi vesicles have the Osmophobic (I) spaces inside and Osmophillic rings outside. A few secretory droplets appear within the Osmophobic spaces, whilst large number of them appear in contact with the Osmophillic rings outside. Mann-Kopsch. × 900. Fig. 14. Transverse section of the liver of Ichthyophis showing Golgi bodies (filamentous, lightly bent and juxta-nuclear) and granular mitochondria (M) (scattered throughout the cytoplasm) in the cortical cells. Mann-Kopsch. × 1800.

In the hepatic cells in Fig. 12 b a few secretory droplets (S) are seen and there is a slight but noticeable change in the structure and position of the Golgi apparatus; while some of them are still rod-like, others have become filamentous. Some of the Golgi rods have assumed crescentic shape (G₃), whilst a few others have become U-shaped forming the Golgi canals (G₄). The protoplasm between the two arms of the bent Golgi apparatus is stained darker than elsewhere. The juxta-nuclear position of the Golgi apparatus seen in the resting cells is gradually lost. The small lateral processes which have become more pronounced fuse with the neighbouring ones and eventually form the typical Golgi network. The secretory droplets (S) make their appearance in close association with the Golgi body. In the hepatic cells in Fig. 13 the secretogenous zone has become more pronounced owing to an extensive development of the Golgi network and an increase in the number of secretory droplets.
It is observed that the strands of the Golgi network are swollen in places to form the Golgi blebs (12 a, 12 b, G.B.), which become transformed into Golgi vesicles (G.V.). The formation of the Golgi vesicles has been recorded by Parat in the intestinal gland cells of Triton, and by Subramaniam in the liver cells of Rhacophorus. It is observed that within the osmophobic space of the Golgi vesicles some secretory droplets are present, whilst a relatively large number of them occur on the osmophilic border of the vesicle outside. The secretory droplets may be observed to have migrated towards the bile capillary yielding place to new ones to be formed.

The Golgi apparatus in the cortical cells is filamentous and juxta-nuclear in position. Some of the filaments are straight, others are slightly bent. A few granular mitochondria are irregularly scattered throughout the cytoplasm of the cell. The secretory droplets were not noticed in my preparations (Fig. 14).

![Diagram](image)

Fig. 15 a. Transverse section of the Gall Bladder (Ichthyophis) showing the three layers, viz., (1) Serosa, (2) Muscularis and (3) Mucosa. The folds of the Mucosa layer are small. Bouin × 50. Fig. 15 b. A part of the above magnified. The mucosa layer has columnar cells with basal nuclei. × 800.

**Discussion**

Widely differing views have been expressed by cytologists regarding the part played by the cytoplasmic inclusions—Mitochondria and Golgi apparatus of glandular cells. Bowen, Cramer and Ludford, Ludford and Nassonow have noticed secretory granules both inside and in relation with the outer border of the Golgi apparatus. Nassonow concludes that the function of the Golgi apparatus is a selective concentration of definite substances,
and Ludford is of opinion that the synthesis of enzymes occurs at the mitochondrial–cytoplasmic interface, and the Golgi apparatus plays only a physical part in condensing the products elaborated by the enzymes secreted by the mitochondria. Duthie\textsuperscript{6} maintains that pro-zymogen granules are first formed in contact with mitochondria, and later they pass on to the Golgi zone and become transformed into zymogen. Subramaniam\textsuperscript{18} states that the appearance of Golgi vesicles in the Golgi strands during the secretory activity of the liver cells is highly significant. My observation shows that a definite \textit{secretogenous zone} is differentiated during the secretory activity of the liver cells. The Golgi network spreads gradually throughout this zone and at the height of the secretory activity the complicated network is very extensively developed. The strands of the Golgi network show the differentiation of Golgi vesicles with the osmophobic spaces inside and osmophilic rims outside. Secretory droplets appear both within the osmophobic space and in close contact with the osmophilic rims outside, and the latter condition is more frequent. In the former case, the secretory droplets make their exit into the cytoplasm either by osmosis through the osmophilic rims or by the rupture of the Golgi vesicles.

The changes exhibited by mitochondria indicate that they also play an important part in the secretory activity. In the resting hepatic cells, the granular mitochondria are irregularly scattered throughout the cytoplasm and form a "mitochondrial film". Their activity is intense in the beginning of the secretory activity when they become differentiated morphologically in the \textit{secretogenous zone} and apparently stimulate the process of secretion. Here they probably act as enzymes and as stated already, initiate the process of secretion. It is important to note that in the actual process of secretion the mitochondrial activity is overshadowed by the Golgi apparatus. The view that mitochondria are enzymatic in nature receives support from the observations, among others, of Horning\textsuperscript{7} and of Subramaniam.\textsuperscript{18} Further the observations recorded in this communication confirm the views put forward by Pollister,\textsuperscript{15} Ludford,\textsuperscript{8, 9} Subramaniam\textsuperscript{18} and others that the Golgi apparatus plays a notable part in the actual synthesis of the products destined to form the secretory substance, and in this important role it is aided by the "Enzymatic activity" of the mitochondria.

\textbf{Summary}

1. The microscopic structure of the liver is described, and that the liver of Apodan forms studied exhibits regional differences into cortical and medullary regions is recorded. That the cortical cells possess polymorphic nuclei is noted. Although amitotic division of the hepatic cells may be the
rule, the occurrence of mitotic activity in the cortical as well as medullary hepatic cells is noted.

2. The nature of the cytoplasmic inclusions—Mitochondria and Golgi bodies (in the normal well nourished forms) is recorded.

3. The fact that the Golgi apparatus is closely associated with the secretory activity is emphasised. The nature of the mitochondrial activity is discussed.

**KEY TO THE LETTERING**

| b.can. | Bile-Canaliculus (Collateral) canal. | L.H.D. | Lumen of the hepatic duct. |
| C.D. | Cystic duct. | Lr. | Liver. |
| Cor. | Cortex. | M. | Granular mitochondria. |
| C.Per.L. | Cell of the peritoneal layer. | M2. | Filamentous mitochondria (long and thin). |
| Comm.tiss. | Connective tissue. | M.C. | Mitochondrial cap. |
| D. | Duodenum. | M.H.D. | Main Hepatic duct. |
| D.C. | Ductus Choledochus. | Mus.L. | Muscular layer. |
| G. | Discrete Golgi bodies. | N. | Nucleus. |
| G.V. | Golgi vesicle. | Per.L. | Peritoneal layer. |
| G.B. | Gall Bladder. | Sec.Z. | Secretogenous zone. |
| H.C. | Hepatic cell. | S. | Secretery mass (Secretory droplets). |
| H.S. | Hepatic Sinusoid. | S1. | Protoplasm in the secretogenous zone which is deeply stained. |
| H.D. | Large hepatic duct. | St. | Stomach. |
| h.d. | Small hepatic duct. | | |
| I. | Osmophobic space within the Golgi vesicle. | | |
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