Reviewing the structure and function of the scorpion’s hepatopancreas

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Abstract
During a long-terms study of scorpions, it was possible to follow changes taking place in the mass, water and lipid content of the hepatopancreas in freshly caught *Scorpio maurose fuscus*. Cyclic events in the female’s reproductive state were shown to be affected by the hepatopancreas. There was a high variability among scorpions in the concentrations of metals in the hepatopancreas. The significance of the hepatopancreas in the scorpion, as a multifunctional organ for both storage and filtering constituents, is reviewed and discussed, and various future avenues of research are outlined.

Keywords Scorpiones; Scorpionidae; *Scorpio*; hepatopancreas; scorpion reproduction; metal content.

1 Introduction
The use of the term hepatopancreas (i.e. midgut gland, digestive gland or liver) in invertebrates remains controversial. In an early study by Guiyesse (1908), he identified two types of cells in the hepatopancreas of the scorpions *Buthus occitanus* (Amoreaux, 1789), and *Buthus europaeus* (Simon, 1879). One kind of cell appears to be largely concerned with absorption, whereas the other with storage.

The earliest detailed study on the cell types in the hepatopancreas of *Scorpio maurose* (Linnaeus, 1758), is by Pavlovsky and Zarin (1926). They review previous literature on this subject. In their micrographs (Figs. 23, 24 there) they depict two cell types based on their structural affinities and named them: “Resorption cell” and “Ferment cell”.

Perhaps Jordan (1912) was first to discuss the physiological functions of this organ trying to examine the question of this double function (what he termed “Die Leberfrage”). There he discussed the nature of this absorption and storage organ in invertebrates. The subject was reviewed later by van Weel (1974) who suggested the inappropriate use of the term liver since in invertebrates the hepatopancreas lacks major functions of that organ. Later, Cornelius (1985) suggested that in invertebrates these glands are primarily exocrine, hence more similar in function to the hepatopancreas.

In a physiological study on *Palamnaeus fulvipes* (Koch, 1837), Bardi and George (1943) described the enzymatic function of the organ. They described the main cell types: the large absorptive cell and the smaller “fermentative” cells. These cell types were later depicted in Plate VIII, Figs. 55, 58 (Abd El-Wahab, 1952).

Awati and Tembe (1956) illustrate the two main cell types: the resorption cell and the ferment cell of the hepatopancreas in *Buthus tamulus* (Fabricius, 1798) (see Fig. XXI there).

Goyffon and Martoja (1983) were first to study the ultrastructure of the scorpion hepatopancreas (i.e. “liver”) in *Androctonus australis* (Linné, 1758). Their cytochemical analysis revealed the “Basophilic cells”
that secrete exoenzymes, and “Digestive cells” where intracellular digestion and storage of glycogen, lipids and mineral salts take place. The waste products (guanine, uric acid, and minerals) are concentrated in ‘Brown body vacuoles’ and are ejected into the lumen (see Figs.14-17 there).

Recently Zouari et al. (2006) localized lipase activity intra-cellular inside granules located in specific vesicles of the hepatopancreas’ digestive cells of *Scorpio maurus*.

Warburg et al. (2002) studied seasonal changes in the microscopic and structure and ultrastructure of the hepatopancreas cells in *Scorpio maurus fuscus* (Ehrenberg, 1829) (Plates 1-4 there). Numerous lipid droplets, pinocytic vesicles, metabolite and heavy metal inclusions can be distinguished in the different cells.

In the present study new information on structure and function of the hepatopancreas is provided and the present knowledge on this organ in scorpions is reviewed and discussed.

### 2 Materials and Methods

Scorpions were collected for 25 years (1974-1999) largely in northern Israel they were hand-picked from under stones, logs and other refuge during day, and with an ultraviolet lamp on suitable moon-less, windless nights (Warburg, 1997). Animals were brought back to the laboratory, sexed and weighed. Since other research on the reproductive cycle needed sectioning animals, their hepatopancreas was excised and separated from the ovariuterus (for details see Warburg et al., 2002). It was then weighed on an electronic balance at ±0.01mg.

For light microscopy (LM) tissue was fixed in Bouin (24-48 hrs) and Altmann (2 hrs), embedded in paraffin, sectioned and stained using Barrett and Azan.

For scanning electron microscopy (SEM): the entire tissue was fixed in 1% Glutaraldehyde in phosphate buffer solution (PBS) at 480C, washed in PBS, and transferred to 2% Guanidin HCl and 2% tannic acid, 1:1 in PBS. The tissue was then post-fixed in 1% OsO4 in PBS for 1 hr, dehydrated in ethanol series for 20 min each, and in 25%, 50%, 75% and 100% Freon 113 for an additional 20 min each. After air drying the tissue was coated in gold and studied in a JEOL T-300.

For transmission electron microscopy (TEM) tissue was fixed in 2% Glutaraldehyde and Cacodylate buffer 0.05m M, and post-fixed in1% OsO4 1.5% Potassium ferrocyanide. Semi-thin sections (2.5µm) were stained in Toluidine blue. Observation was by JEOL-100-B (details in Warburg et al., 2002).

The presence of lipids in the hepatopancreas was demontrated by using the histochemical methods of staining sections with either Oil-Red-O or Sudan 3. Qu antitative measurements of lipids were done by placing the hepatopancreas in chloroform:ethanol solutions and after double centrifugations the supernatant was dried and weighed.

The concentration of four metals (Cu, Cd, Ni, Pb) was measured in the hepatopancreas of male *S. m. fuscus* using Emission Spectrometry Optima 3000-I.C.P.

### 3 Results and Discussion

#### 3.1 The structure of the hepatopancreas in scorpions

The hepatopancreas of *Scorpio maurus fuscus* is lobed (Fig.1, Fig. 2 A-C). It envelopes the ovariuterus as seen in a ventral aspect (Fig.1 B, C). The lobes are less prominent in its dorsal aspect (Fig.1A). Two cell types can be distinguished: basophilic (ba) and digestive (di) (Fig. 3 A-C). Sections through the hepatopancreas’ lobes show both lipid globules and droplets (L).

#### 3.2 The hepatopancreas as a reserve organ

Our present knowledge of hepatopancreas chemistry is based on seventeen studies of nine scorpion species belonging to two families. Most of these studies were conducted on a single scorpionid species (Tables 1, 2).
**Fig. 1** Exposed hepatopancreas of *S.m. fuscus* under a dissecting microscope. A- dorsal view (scale bar 1mm). B-ventral view, arrow indicated embryonic diverticulum during early embryogenesis, Lo-lobes (scale bar 1mm). C-a depleted hepatopancreas with well developed embryos (arrow) inside embryonic diverticulae prior to parturition (scale bar 1mm).

**Fig. 2** SEM view of the scorpion hepatopancreas. Lobes (Lo). A- (scale bar 100µm); B- (scale bar 100µm); C- (scale bar 1µm).
Table 1 Scorpion species in which biochemical investigation on the hepatopancreas were conducted*

<table>
<thead>
<tr>
<th>BIOCHEMISTRY</th>
<th>KEY TO SPECIES</th>
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<th>KEY TO SPECIES</th>
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<tr>
<td>Diastase</td>
<td>B</td>
<td>Total Lipid</td>
<td>A, E, I</td>
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<td>B, F</td>
<td>HIS</td>
<td>D</td>
</tr>
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<td>Pepsin</td>
<td>B, F</td>
<td>Free Amino acids</td>
<td>A</td>
</tr>
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<td>Trypsin</td>
<td>B, F</td>
<td>Glycogen</td>
<td>A, D, E, F, H</td>
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<td>Glucose</td>
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<td>B, F</td>
<td>Fructose</td>
<td>H</td>
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<tr>
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</tr>
<tr>
<td>Catalase</td>
<td>B, F</td>
<td>Pyruvic acid</td>
<td>H</td>
</tr>
<tr>
<td>Total Protein</td>
<td>E</td>
<td>Malic acid</td>
<td>H</td>
</tr>
<tr>
<td>TNPS</td>
<td>D</td>
<td>Succinic acid</td>
<td>H</td>
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*A-Buthus tamulus (Buthidae); B-B. eupeus (Buthidae); C-B. quinquestriatus (Buthidae); D-Androctonus australis (Buthidae); E-Heterometrus scaber (Scorpionidae); F-Heterometrus fulvipes (Scorpionidae); G-Palamaeus fulvipes (Scorpionidae); H-Palamaeus bengalensis (Scorpionidae)
### Table 2: References in which biochemical investigation on the hepatopancreas was conducted*

<table>
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<th>Key to Sources</th>
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<tr>
<td>Pepsin</td>
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<td>2</td>
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<td>Trypsin</td>
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<td>Free Amino acids</td>
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<td>Glycogen</td>
<td>2,4,9,10,11,12,14</td>
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<tr>
<td>Lipase</td>
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<td>Glucose</td>
<td>2,10,13,14</td>
</tr>
<tr>
<td>Inulase</td>
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<td>Fructose</td>
<td>13</td>
</tr>
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<td>Mannose</td>
<td>13</td>
</tr>
<tr>
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<td>Sucrose</td>
<td>13</td>
</tr>
<tr>
<td>Phosphorylase</td>
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<td>Pyruvic acid</td>
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<tr>
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<tr>
<td>TNPS</td>
<td>2</td>
<td>Succinic acid</td>
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The role of the hepatopancreas in enabling digestion by the production of a series of enzymes on the one hand, and storing nutrients on the other, was quite clearly demonstrated. Thus, the role of the scorpion hepatopancreas as a storage organ for glycogen was shown by Ramamurthi et al. (1975). Chengal Raju, Govindappa and Swami (1978) found a difference in Glycogen between male and female *Heterometrus fulvipes* (Koch, 1837). The concentration of glucose in the hepatopancreas of *H. fulvipes* was 40.2 % greater in normal females compared to embryo-bearing ones (Jayaram et al., 1978). Subburam and Gopalakrishna (1978) found in the same species that hepatopancreas glycogen increases during embryogenesis, declining until parturition. These two observations indicate that both glucose and glycogen are being utilized during embryogenesis.

Raghavaiah and Ramamurthi (1977) have demonstrated that the hepatopancreas of *H. fulvipes* has the highest lipid concentration. These lipids are stored in the embryo during gestation as was demonstrated by Subburam and Reddy (1978). Lipids provide vital energy source during embryogenesis Rajyalakshmi and Umamaheshwar (1992). The gonads have a high rate of lipid uptake and accumulate lipids (Raghavaiah and Ramamurthi, 1977).

The presence of lipids in the hepatopancreas was demonstrated by using the histochemical methods of staining sections with either Oil-Red-O or Sudan 3 (see L and arrowheads in Figs. 4, 5). In addition, the hepatopancreas was placed in chloroform:ethanol solutions and after double centrifugations the supernatant was dried and weighed. The lipid content (mass) of males was found to be lower than that of female *S.m. fuscus* (Fig. 6).
**Fig. 4** Sections through hepatopancreas lobes (L) showing lipid droplets (arrow heads) scale bars 100 μm.

**Fig. 5** Showing lipid droplets (arrow heads). a- scale bar 1μm; b- (Barrett) scale bar 1μm; c- scale bar 1μm.
3.3 The hepatopancreas under stressful conditions

The role of hepatopancreas in storing glycogen and fat which is utilized during starvation is discussed by Van Weel (1974). Continuous fasting (deprivation of food for more than three days) induced depletion of the hepatopancreas reserves (Goyffon and Martoja, 1983). Rajyalakshmi and Umamaheshwar (1992) described in *H. fulvipes*, the depletion of hepatopancreas as evidence of utilization of stored lipids under stress conditions. This was demonstrated in *Palamnaeus* (now *Heterometrus* bengalensis) (C.L.Koch), by Sinha (1982) and Sinha and Kanungo (1967) who found that the glycogen within the hepatopancreas decreased with progressive starvation. Digestion time in *Paruroctonus utahensis* (Williams, 1968) was shown to be 72 hrs (Bradley, 1982).
3.4 The role of the hepatopancreas in effecting the reproductive cycle

Although it was suspected by previous researchers, no proof was available of the hepatopancreas significance in scorpion reproduction prior to a recent study on S.m. fuscus (Warburg et al., 2002). Seasonal changes in S.m. fuscus hepatopancreas mass and its percentage out of the body mass are shown in Fig. 7a, b. Both the hepatopancreas mass (Fig. 7a), and its percentage per body mass (Fig. 7b), increased significantly from autumn to summer spring (this is the period of embryogenesis), and decreased towards parturition. There is a positive relationship between the hepatopancreas’ mass and the number of large diverticulae in S.m. fuscus. The percentage of PPDs (post-partum diverticulae) is related to the parturition period during summer and fall.
and thus increases towards summer (Fig. 8). The scorpion female reproductive system has been recently reviewed (Warburg, 2010).

Mathew (1948) has shown that during gestation the liver (i.e., hepatopancreas) of Palamnaeus (now Heterometrus) scaber (Thorell, 1876) accounts for an average of 15% of the body mass (this drops to 7% after parturition).

3.5 The role of the hepatopancreas as a waste-storage organ
In the scorpionid S. m. fuscus hepatopancreas water content ranged between 14.3% during winter to 25.7% in spring (Figs. 9A, 10A). In the buthid Leiurus quinquestritus (H & E 1829), it ranged between 14.8% in summer to 18.3% during autumn (Figs.9B, 10B). Finally, in the buthid Hottetotta judaica (E.Simon, 1872), it ranged between 11.8% in summer to 28% in spring (see Warburg 1986, & Figs.8C, 9C here).

The hepatopancreas water stores are apparently used during dehydration in order to replenish haemolymph water (Gefen and Ar, 2005). The percentage of the hepatopancreas’ water content in S. m. fuscus varied seasonally ranging between 47.8 to 68.4% of its mass (Warburg et al., 2002).

The hepatopancreas is the main organ which is capable of storing heavy metals. These are stored as spherocrystals in vacuoles. The concentration of four metals was measured in the hepatopancreas of male S. m. fuscus. The concentration was related to the scorpion’s mass (Fig.11). The metals tested were: Copper, Zinc, Cadmium and Lead. The last two metals are considered toxic elements indicative of pollution in the habitat. Both Copper and Zinc are needed for metabolism and their concentration was considerably higher than the first two. Concentration of Copper averaged 18.48 µmol/g dry mass (ranging between 7.45-36.5 µmol/g), and that of Zinc averaged 11.33 µmol /g dry mass (ranging between 3.77-18.9 µmol/g). Concentration of both metals varied greatly (five folds) indicating the high individual variability in their accumulation in the hepatopancreas regardless of the scorpions’ mass. Both toxic metals (Cd and Pb) though present in much lower concentrations in the hepatopancreas, showed higher variability in their concentrations (6.5 and 7 folds).
Fig. 9 Seasonal changes in hepatopancreas water contents (%). a. In *S. m. fuscus*. b. In *L. quinquestriatus*. c. In *H. judaica.*
Fig. 10 Seasonal changes in hepatopancreas water contents out of total water contents. a. In *S. m. fuscus*. b. In *L. quinquestriatus*. c. In *H. judaica.*
Fig. 11 Metal concentrations of copper, zinc, cadmium and lead in the hepatopancreas of male *S. m. fuscus.*
4 Future Avenues of Research

The ultrastructure and ultra-cytochemistry of the scorpion hepatopancreas were hardly ever studied. There is a need to characterize the organelles of the different cells and prove their function using modern methods. The dynamical changes in the cells inclusions need to be followed and correlated experimentally with physiological processes. It will need extensive biochemical research and microscopical examination of dynamical changes in the two main cell types, under experimental conditions.

Likewise, the significance of the hepatopancreas both as a reserve organ for lipids and glycogen utilized during gestation, needs to be proven experimentally. One suggested avenue of research is and morph-ecological approach relating cell ultrastructure with moulting or oogenetic cycles. Another approach, a morpho-physiological approach would relate water economy or starvation with micropscopical cellular changes. Finally, the role of the hepatopancreas in storing and detoxificating metals needs to be studied experimentally. Until the exact physiological activity of that organ can be clarified, it would be still appropriate to continue using the term hepatopancreas.

Scorpions are particularly interesting in that they are a top predator among invertebrates accumulating toxic products of their prey. The main questions to be addressed are: What is the exact topography within the hepatopancreas, of each metal? Do they all accumulate inside vesicles and are excreted later into the follicle lumen? Or are they located inter-cellular? Is there a way that metal concentration can drop during the scorpion’s lifetime? Is there any toxic effect on the scorpion’s well-being, fertility or longevity?

Further questions to be addressed:

Does a well-stocked hepatopancreas enable production of more or larger offspring, as compared to a decimated hepatopancreas? To what extent does the hepatopancreas condition affect gestation? Can it cause disruption of oogenesis, or vitellogenesis? Can it cause embryo resorption (Warburg, 2012).

Moreover, will it be possible to identify past stress encountered by the female, through examining the state of the hepatopancreas?

It would be of great advantage if a biopsy technique can be worked out in order to enable analyzing hepatopancreas samples at intervals without needing to sacrifice the scorpion.

All our present knowledge of hepatopancreas’ chemistry (reviewed here) is based solely on 17 studies of nine enzymes, three amino acids, lipid and protein components all conducted on nine scorpion species belonging to two families. It is therefore essential to broaden our knowledge on both the chemical components and the number of scorpion species in order identify a baseline before attempting any experimental work. Both hepatopancreas mass (as percentage of body mass), and its water content (as percentage out of total water content), show significant seasonal changes. Consequently, it would be best to investigate both a saturated and a depleted hepatopancreas. These conditions can be controlled experimentally. Some scorpion species that breed in captivity would be ideal for this study (like Pandinus imperator (Koch, 1841), Liocheles australasiae (Fabricius, 1775). The isolated female scorpions need to be kept under energetically known diet conditions. Two such different diets can be used to enable comparison.

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References


Warburg MR. 1986. The distribution of water in the body compartments of four scorpion species. Comparative Biochemistry and Physiology, 84A: 637-641


Warburg MR. 2010. The scorpion female’s reproductive system; a partial review. Anatomical Records, 293-1738-1754

