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Effect of hypoxia on tissue metabolism of midgut gland of the scorpion *Heterometrus fulvipes*

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Abstract. The function of the midgut gland of the scorpion *Heterometrus fulvipes* has been investigated in relation to hypoxia. Regional differences in the midgut gland became apparent, one part being more active metabolically than the other. It is concluded that the midgut gland might be serving as the liver, as gluconeogenesis is predominant.

Keywords. *Heterometrus fulvipes*; midgut gland; hypoxia; tissue metabolism; gluconeogenesis.

1. Introduction

The gland which performs the function of a 'liver' in crustaceans (Vonk 1960) is referred to as the "hepatopancreas" inspite of lack of enough evidence to support its hepatopancreatic function (Phillips *et al* 1977). Van Weel (1974) has questioned the usage of the word hepatopancreas for this gland and called it the midgut gland (MGG).

The phylogenetic origin of MGG in the members of the phylum Arthropoda is puzzling. The presence of a conspicuous digestive gland in the non-tracheate Arthropods of classes Crustacea and Arachnida, its replacement by a few to many digestive caecae (hepatic or midgut caecae) in the tracheate insects and its total absence in the tracheate Myriapods prompted us to investigate the nature and function of this MGG in the scorpion *Heterometrus fulvipes* in relation to respiration.

2. Material and methods

Active *H. fulvipes* females (4·5–6·5 g) in the non-breeding season were used. The MGG was divided into three parts—anterior (I), middle (II) and posterior (III) in order to know the regional differences if any. The oxygen consumption and carbon dioxide production in all parts was studied using the conventional Warburg apparatus as given by Umbreit *et al* (1959) and their RQ values calculated.

Fuels, metabolites and end products (glycogen, phospholipids, reducing sugars, lactate and pyruvate) were estimated in the anterior and posterior parts of the MGG and the in vitro effect of hypoxia (30 min) on their levels evaluated at 37°C.

Cyanide method of Park and Johnson (1949) was used to estimate reducing sugars. Glycogen was estimated after ethanol precipitation by the method of Good *et al* (1933); Barker and Summerson (1941) method was adopted for lactate estimation. The method

of Youngburg and Youngburg (1930) for phospholipid and the method of Lu (1939) as given by Umbreit *et al* (1959) for pyruvate were employed. Total carbohydrates were estimated by the colorimetric method of Carrol *et al* (1956).

3. Results and discussion

Although there appears to be no difference in oxygen consumption or carbon dioxide production in the three parts of the MGG, RQ values appear to be markedly different, (table 1). Therefore RQ has a different value for each of the major food components and serves to determine what substances are being burned (Oser 1954). MGG I with RQ value of more than 1 is involved probably in the interconversion of carbohydrates and fats.

Table 1. Regional differences in O_2 consumption and CO_2 production in the midgut gland.

Regions of midgut gland	O_2 uptake (μl/gm wet wt/10′)	CO_2 liberation (μl/gm wet wt/10′)	RQ value
MGG I	71·51 (12) ±24·89	91·31 (12) ±36·42	1·28
MGG II	68·99 (12) ±20·52	71·57 (12) ±19·71	1·04
MGG III	108·38 (12) ±59·34	99·21 (12) ±34·95	0·915
	t = 1·99 (MGG I *vs* III)	t = 0·54 (MGG I *vs* III)	

t value from table = 2·07 (5%).
Numbers in parentheses denote the number of samples studied.

Table 2. Water and protein contents in the midgut gland.

Regions of midgut gland	Percentage of water	Protein content (mg/g wet wt)
MGG I	53·50 (6) ±5·45	72·26 (6) ±47·16
MGG II	56·95 (6) ±4·78	56·13 (6) ±15·80
MGG III	60·49 (6) ±16·60	155·33 (6) ±62·25
	t = 0·98 (MGG I *vs* III)	t = 2·61 (MGG I *vs* III)

t value from table = 2·23 (5%).
Numbers in parentheses denote the number of samples studied.

Table 3. Levels of glucose, glycogen, lactate, total carbohydrate and phospholipids in different regions of the midgut gland under different conditions.

Particulars	Prior to incubation				After incubation (30″)			
	MGG I		MGG III		MGG I		MGG III	
	Normal	Hypoxic	Normal	Hypoxic	Normal	Hypoxic	Normal	Hypoxic
Glucose (mg/g wet wt)	31·66 ±15·87	32·68 ±13·75	35·84 ±2·76	27·35 ±5·70	27·28 ±11·73	13·51[d] ±6·36	19·52[f] ±9·44	26·70 ±11·198
Glycogen (mg/g wet wt)	5·51 ±3·17	4·73 ±3·19	4·48 ±1·79	5·19 ±2·50	4·33 ±2·72	6·52 ±3·25	2·70 ±1·79	7·70 ±5·66
Lactate (mg/g wet wt)	10·93 ±9·005	6·97 ±4·83	7·35 ±5·20	8·96 ±6·70	5·89 ±4·17	5·09 ±3·08	6·28 ±3·75	5·00 ±3·94
Total carbohydrates (μ mol of glucose/g wet wt)	240·62 ±53·29	367·78 ±107·01	527·07[a] ±101·14	538·67[b] ±94·99	404·92[d] ±105·79	581·59[e] +198·67	640·30[c] ±119·79	634·89 ±72·42
Phospholipids (mM of phosphorus/g wet wt)	81·77 ±16·08	83·56 ±24·37	65·53 ±15·59	82·51 ±19·10	82·81 ±15·95	94·48 ±15·03	85·98[f] ±16·09	85·04 ±13·10

Symbols indicate that the samples are significant at 5% level.
Number of samples studied = 6.

[a] Prior to incubation MGG I *vs* MGG III Normal
[b] Prior to incubation MGG I *vs* MGG III Hypoxic
[c] After incubation MGG I *vs* MGG III Normal
[d] Preincubation *vs* Post incubation MGG I Normal
[e] Preincubation *vs* Post incubation MGG I Hypoxic
[f] Preincubation *vs* Post incubation MGG III Normal

MGG II with RQ value of 1 might be carbohydrate oriented in its metabolism. MGG III with RQ value of 0·9 might be metabolising non-carbohydrates or mixed fuels. MGG III with a significantly high protein content (table 2) might be the seat of high synthetic or secretory activity.

Table 3 shows that MGG I loses glucose under hypoxia whereas MGG III maintains more or less the same level as under normal conditions. Apparently under hypoxia, glucose production is continuing in MGG III, but not in MGG I. The drop in glycogen levels in MGG III under normal conditions and the higher than the normal hypoxic levels of glycogen probably further suggests the presence of glycogen synthetic activity in MGG III. Phillips *et al* (1977) are of the opinion that the hepatopancreas of the crustacean *Homarus gammurus* may not be the site for gluconeogenesis. In vivo studies reveal gluconeogenesis taking place from lactate in *Cherax destructor* (Phillips *et al* 1977). Munday and Poat (1972) suggested MGG as a possible site for gluconeogenesis. Giles *et al* (1975) have also expressed a similar view. Maintenance of more or less the same glucose level under hypoxic conditions in the scorpion MGG might be due to the gluconeogenetic role of MGG III and gluconeogenesis is therefore one of its important functions as in the vertebrate liver.

The absence of any change (table 3) in phospholipid content on incubation and lack of any difference in MGG I and MGG III might indicate the membranous nature of the gland and these lipids may get involved only on prolonged starvation of the animal (Reddy and Selvarajan 1975; Sinha and Kanungo 1967). The unexpectedly high levels of these phospholipids in MGG as compared to the levels of total carbohydrate, glycogen, glucose or lactate, probably suggest the role of the MGG as a storage organ for these lipids. Similar situation seems to prevail in other arthropods as well (Ravindranath Gupta 1971; Satyam 1976; Venkata Reddy 1976). The liver mainly functions as a storage organ for glycogen and lipids. The digestive gland of malacostracan crustaceans contains glycogen and fat. The stored glycogen is said to be utilized during the formation of new chitinous substances (Scheer 1957) and during exercise in insects (Clements 1955). But storage of glycogen alone cannot justify the term 'liver' for this organ. However, since it also has a gluconeogenetic role it could be suggested that the MGG of the scorpion serves as a liver.

The higher levels of glucose, glycogen and phospholipid in MGG I when calculated per milligramme protein (tables 2 and 3) together with the low protein value and lack of difference in their levels between MGG I and III when calculated on wet weight basis may indicate synthesis of these fuels in the protein rich MGG III and probably their storage in the protein deficient MGG I.

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