CHAPTER II
RESPIRATION
INTRODUCTION:

Metabolic rate is usually measured by the rate of oxygen consumption. Further, the rate of respiration has been identified as an index of stress in organisms exposed to altered thermal regime (Saroja, 1962; Prosser, 1973; Hazel and Prosser, 1974; Das, 1984). Inspite of voluminous data on respiration in thermally acclimated animals, little is known about the mechanisms involved in its regulation. It is a well known fact that oxygen consumption in crustaceans is regulated by eyestalk hormones (Skorkowski et al. 1974; Raghaviah, 1977; Vasantha et al. 1979; Reddy, 1981; Reddy and Ramamurthy, 1987). Since oxygen consumption regulation is one of the indicators of thermal acclimation, an eyestalk hormone which influences respiration might also be the controlling mechanisms for thermally acclimating animals. Further, it was reported that seasonal differences in respiration of crabs were caused by their past thermal history and these seasonal differences were eliminated on bilateral eyestalk ablation (Vernberg, 1959; Silverthorn, 1973, 1975a). This link between acclimation influenced respiration and neurosecretory system supports the theory that respiration regulating factor is involved in thermal acclimation. But investigations in this field are scarce and hence the present study has been undertaken.
RESULTS:

Different solvents such as distilled water, ethanol, acetone and saline were employed for the extraction of eyestalk principle and their effects on whole animal oxygen consumption were summarized in table 2.1.

Injection of aqueous and ethanolic eyestalk extracts of normal crabs produced appreciable decrease in the rate of oxygen consumption (28.58% and 37.3% respectively) of normal crabs (Fig.2.1). Whereas the administration of acetone or saline eyestalk extract yielded only a meagre response (6.6% and 11.83% respectively) in normal crabs.

As the greater effect of eyestalk principle is found with ethanol extract, ethanol was selected for the preparation of eyestalk extracts for further studies.

Effect of injection of eyestalk extracts from warm and cold acclimated crabs on the whole animal and HP, CLM and GL respiration was presented in table 2.2 and fig.2.2 to 2.5. From the results it is clear that eyestalk extract from cold-acclimated crabs enhanced the respiration of whole animal and the
HP, CLM and GL of normal crabs by 13.49%, 13.83%, 13.93% and 18.37% respectively and the respiration of whole animal and the HP, CLM and GL from warm-acclimated crabs by 12.94%, 13.51%, 13.56% and 18.15% respectively. Whereas the injection of eyestalk extracts of warm-acclimated crabs brought a decrease in the respiration of whole animal and the HP, CLM and GL of normal animals by 10.95% 11.44% 11.50% and 16.02% respectively and the respiration of whole animal and the HP, CLM and GL of cold-acclimated crabs by 10.55% 11.14%, 11.20% and 15.86% respectively when compared to the respiration of control crabs.

DISCUSSION:

Presence of respiratory inhibiting principle was well demonstrated in the eyestalks of *O. senex senex* (Raghaviah, 1977; Reddy, 1981; Reddy and Ramamurthi, 1987) and greater effect of ethanol eyestalk extracts was noticed earlier by Rao et al. (1968), Silverthorn (1975b), Reddy (1981) and Sreenivasamurthy (1985).

Pioneering investigations of Rao and his associates (Rao et al. 1967, 1968; Bartell et al. 1971) on the differences in the pigment dispersing hormone and Raghaviah (1977) and Reddy (1981) on the respiratory depressing hormone activities with different
extraction media lead to the conclusion that the high activity of ethanol extract was due to micellar lipoidal material in complex with active polypeptide eyestalk component. Rehm (1959) reported some histological evidence that in the sinus gland of the crab, Carcinus maenus, a lipoidal material forms a part of the neurosecretory system. Further these authors reported that the loss of activity with saline and acetone extracts may be due to dissociation of active polypeptide complex.

Another fact that requires closer scrutiny is the relatively low activity of respiration inhibiting principle of aqueous extract as compared with ethanolic extract. The low activity with aqueous extract was reported by Bartell et al. (1971) for melanin dispersing hormone. These authors obtained some histological evidences for low activity evoked by aqueous extract and observed limited fragmentation of neurosecretory granules in the aqueous eyestalk extract and they concluded that the low activity was presumably due to slow release of high active polypeptide material from them.

In view of the above literature insights which are in agreement with the results of present study, it can be postulated that the active eyestalk principle in O. senex senex also may be
protenaceous associated with lipoid carrier substance (Vide Chapter-V).

From the results presented in Table 2.2 it is clear that eyestalk extracts from cold-acclimated crabs enhanced the respiration of the whole animal and tissue respiration of normal and warm-acclimated crabs. Where as the eyestalk extracts from warm-acclimated crabs decreased the whole animal and tissue respiration of normal and cold-acclimated crabs.

From the above results it is clear that respiration in thermally acclimated crabs is under the control of eyestalk factors. Further it appears that there are two distinct factors regulating the changes in oxygen consumption in thermal acclimated crabs. One factor is present in warm-acclimated crabs and lowers oxygen consumption while the other is present in the cold-acclimated crabs and enhances respiration. This is in concurrence with the reports of Rao (1968) and Kale and Rao (1973). These authors reported the existance of two distinct hormones in the body fluids of cold-acclimated and warm-acclimated earthworms, Lampito mauritii. They found that the addition of body fluids of cold-acclimated worms increased the in vitro respiration of tissues of warm-acclimated animals and in a reciprocal study, the body fluids of warm-acclimated worms added
to cold-acclimated tissues depressed the oxygen consumption. These authors speculated that the blood borne substances were hormones and named as "Respiratory enhancing hormone" (REH) and "Respiratory depressing hormone" (RDH) respectively. This was supported by their observation of neurosecretory cells. The neurosecretary activity, some times, the number of cells were greatly increased in cold-acclimated worms. In warm-acclimated animals the position of neurosecretory cells was different from that in the cold-acclimated animals. Similar changes in neurosecretory cells on thermal acclimation was also reported in arthropods and other invertebrates (Lomte and Barhanpurkar, 1979; Ushasharma et al. 1980).

Presence of REH and RDH were also reported in fiddler crab, *Uca pugilator* (Silverthorn, 1973, 1975a,b) and in fresh water crab, *Paratelphusa jacquemontii* (Kulkarni and Kamath, 1983). These authors reported that these hormones are synthesized and released from X-organ and sinusgland complex of eyestalks.

The mode of action of these hormones is still not known. Many physiological functions are affected during cold acclimation, REH may also influence many physiological process.

An increase in the flow of material through glycolytic
pathway would increase respiration. A hormone mediated substrate increase in glucose would cause increased activity of glycolytic enzymes and would result in increased oxygen consumption. Accordingly an increase in the activities of glycolytic enzymes was noticed on injection of eyestalk extracts from cold-acclimated crabs. (Vide Chapter III).

Hormonal regulation of these enzymes might be accomplished in several ways: REH may induce messenger RNA (m-RNA) synthesis (Jacob and Monod, 1961). Increased RNA levels would in turn result in more enzyme synthesis and overall increase in enzyme activity (Rao, 1968; Silverthorn, 1975b). Accordingly in the present study eyestalk extract from cold-acclimated crabs increased RNA synthesis (vide Chapter III). Allosteric regulation of enzymes in glycolytic pathway also shown to occur in cold-acclimated animals and this regulation was reported to be by hormones (Rao, 1968, Silverthorn, 1975b). If this is so the opposite will be true in the case of warm acclimation and with RDH.

In the same way in the present study also the increase in respiration brought about by eyestalk extracts from cold-acclimated crabs may be due to the presence of "Respiratory enhancing hormone" and decrease in respiration by eyestalk extracts from warm-acclimated crabs may be due to the presence of
"Respiratory depressing hormone".

The existence of two antagonistic respiration regulating hormones is reasonable from an adaptive viewpoint. Those poikilothermic animals which are exposed to low temperature stress must find a means of elevating their metabolic rate over that indicated by the ambient temperature in order to produce enough energy to survive or to remain active. Conversely, those animals subjected to high temperature stress require some behavioural or physiological mechanisms through which they can lower their metabolic rate and avoid increased requirement for metabolic sources which they are unable to fulfil.
Table 2.1: Effect of injection of eyestalk extracts prepared with different solvents on the rate of whole animal oxygen consumption (ml O₂/h) of O. senex senex. Values are mean ± SD of 62 individual observations.

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>2.456 ± 0.21</td>
<td>1.755 ± 0.18</td>
</tr>
<tr>
<td>% change</td>
<td></td>
<td>-28.58</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>2.564 ± 0.34</td>
<td>1.607 ± 0.23</td>
</tr>
<tr>
<td>% change</td>
<td></td>
<td>-37.34</td>
</tr>
<tr>
<td>Acetone extract</td>
<td>2.287 ± 0.22</td>
<td>2.136 ± 0.21&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>% Change</td>
<td></td>
<td>-6.60</td>
</tr>
<tr>
<td>Saline extract</td>
<td>2.375 ± 0.22</td>
<td>2.094 ± 0.19</td>
</tr>
<tr>
<td>% Change</td>
<td></td>
<td>-11.83</td>
</tr>
</tbody>
</table>
Table 2.2: Effect of eyestalk extracts of cold and warm acclimated crabs on the whole animal oxygen consumption and tissue respiration of normal and experimental crabs *O. senex senex*. Each value is mean ± SD of six individual observations.

<table>
<thead>
<tr>
<th>Oxygen consumption/tissue respiration</th>
<th>Control</th>
<th>Eyestalk extracts of CA-crabs to normal crabs</th>
<th>WA-Control</th>
<th>Eyestalk extract of CA-crabs to WA-crabs</th>
<th>Control</th>
<th>Eyestalk extract of WA-crabs to normal crabs</th>
<th>CA-control</th>
<th>Eyestalk extract of WA-crabs to CA-crabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole animal (ml O₂/animal/h)</td>
<td>2.468</td>
<td>2.801</td>
<td>2.805</td>
<td>3.168</td>
<td>2.465</td>
<td>2.195</td>
<td>2.067</td>
<td>1.849</td>
</tr>
<tr>
<td>% Change</td>
<td>±0.16</td>
<td>±0.29</td>
<td>±0.25</td>
<td>±0.32</td>
<td>±0.16</td>
<td>±0.26</td>
<td>±0.14</td>
<td>±0.15</td>
</tr>
<tr>
<td>Hepatopancreas (µl O₂/g/h)</td>
<td>1.894</td>
<td>2.156</td>
<td>2.162</td>
<td>2.454</td>
<td>1.896</td>
<td>1.679</td>
<td>1.580</td>
<td>1.404</td>
</tr>
<tr>
<td>% Change</td>
<td>±0.08</td>
<td>±0.14</td>
<td>±0.12</td>
<td>±0.15</td>
<td>±0.09</td>
<td>±0.11</td>
<td>±0.08</td>
<td>±0.09</td>
</tr>
<tr>
<td>Claw Muscle (µl O₂/g/h)</td>
<td>2.018</td>
<td>2.299</td>
<td>2.308</td>
<td>2.612</td>
<td>2.017</td>
<td>1.785</td>
<td>1.678</td>
<td>1.490</td>
</tr>
<tr>
<td>% Change</td>
<td>±0.11</td>
<td>±0.15</td>
<td>±0.12</td>
<td>±0.11</td>
<td>±0.13</td>
<td>±0.08</td>
<td>±0.11</td>
<td>±0.11</td>
</tr>
<tr>
<td>Gill (µl O₂/g/h)</td>
<td>2.412</td>
<td>2.855</td>
<td>2.870</td>
<td>3.391</td>
<td>2.409</td>
<td>2.023</td>
<td>1.892</td>
<td>1.592</td>
</tr>
<tr>
<td>% Change</td>
<td>±0.13</td>
<td>±0.17</td>
<td>±0.14</td>
<td>±0.18</td>
<td>±0.14</td>
<td>±0.12</td>
<td>±0.12</td>
<td>±0.14</td>
</tr>
<tr>
<td>% Change</td>
<td>18.37</td>
<td>18.15</td>
<td>-16.02</td>
<td>-15.86</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
LEGEND

Fig. 2.1: per cent difference in the whole animal oxygen consumption of the crab, *O. senex senex* injected with aqueous eyestalk extract (AqE), ethanol eyestalk extract (EE), acetone eyestalk extract (AcE) and saline eyestalk extract (SE).
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Fig. 2.2: Per cent difference in the whole animal oxygen consumption upon injection of cold-acclimated eyestalk extract to normal crabs (CN), cold-acclimated eyestalk extracts to warm acclimated crabs (CW), warm-acclimated eyestalk extracts injected to normal crabs (WN) and warm-acclimated eyestalk extract injected to cold-acclimated crabs (WC).

Fig. 2.3: Per cent difference in tissue respiration of hepatopancreas (HP), upon injection of cold-acclimated eyestalk extract to normal crabs (CN), cold-acclimated eyestalk extracts to warm-acclimated crabs (CW), warm-acclimated eyestalk extracts to normal crabs (WN) and warm-acclimated eyestalk extracts to cold-acclimated crabs (WC).

Fig. 2.4: Per cent difference in tissue respiration of the claw muscle (CLM), upon injection of cold-acclimated eyestalk extract to normal crabs (CN), cold-acclimated eyestalk extracts to warm-acclimated crabs (CW), warm-acclimated eyestalk extracts to normal crabs (WN) and warm-acclimated eyestalk extracts to cold-acclimated crabs (WC).

Fig. 2.5: Per cent difference in tissue respiration of the gill (GL), upon injection of cold-acclimated eyestalk extract to normal crabs (CN), cold-acclimated eyestalk extracts to warm-acclimated crabs (CW), warm-acclimated eyestalk extracts injected to normal crabs (WN) and warm-acclimated eyestalk extract injected to cold-acclimated crabs (WC).