CHAPTER-IV

CYTOCHEMICAL STUDIES ON SOME RESPIRATORY 
ENZYMES IN STYLOCEPHALUS CONOIDES DEV.
INTRODUCTION

Protozoans, whether free-living or parasitic, are well-known for the diversity in their habitats; and as such, they are periodically exposed to different environmental situations. Obviously, they show a variety of biochemical and physiological adaptations. As a result, these organisms are likely to show a wide range of methods of obtaining energy necessary for their diversified biological activities. In a biological organization, energy release or storage is an integral part of the metabolism of carbohydrates, lipids and proteins. Some of the metabolic pathways involve the tricarboxylic acid (TCA) cycle, the pentose cycle and the anaerobic glycolysis. These pathways involve the active participation of some dehydrogenases which can be histochemically identified in organisms. In turn, the cytochemical demonstration of some of the dehydrogenases offers an useful method to detect different pathways involved in energy metabolism.

Studies on the afore-said metabolic pathways, involving cytochemical demonstration of dehydrogenases and cytochrome oxidases in protozoa are recent and limited to a few species.
In flagellates, succinate dehydrogenase (SDH) activity has been demonstrated in *Chilomonas* sp (Notochin and Seravin, 1962); reduced nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) diaphorases and SDH activities have been shown in *Trichomonas vaginalis* (Sharma and Bourne, 1963b), malate dehydrogenase (MDH) activity in the same species has been demonstrated by Tanaka (1970) and Brugerolle et Metenier (1973); in addition, lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) and isocitrate dehydrogenase (ICDH) in this flagellate have also been cytochemically demonstrated by Tanaka (1970). In ciliates, SDH activity has been shown in *Vorticella microstomata* and *Paramecium caudatum* (Natochin and Seravin, 1962); the activities of SDH, MDH, ICDH, LDH, glutamate dehydrogenase (GDH) and \( \alpha \)-glycerophosphate dehydrogenase (\( \alpha \)-GPDH) have been observed in *Opalina carolinensis* (Hunter, 1955), cytochrome oxidase has been observed in *Euplotes eurystomus*, *Stylonychia pustulata* (Hunter 1959a), *Balantidium coli* (Sharma and Bourne 1963a) and in *O. ranarum* (Amoji, 1975). In sporozoans, other than gregarines, such studies have been made on different species of *Eimeria*, *Plasmodium*, *Babesia* and *Toxoplasma*. SDH activity has been observed in *E. intestinalis* (Beier, 1962) and in *E. steidae* (Frandsen, 1970), and in
Toxoplasma gondii (Akao, 1971); activity of IDH has been observed in Plasmodium berghei, P. lophurae and Babesia rhodhaini (Sherman, 1962) and in E. steidae (Frandsen, 1970) and in Toxoplasma gondii (Akao, 1971); in addition, MDH activity has also been shown in T. gondii (Akao, 1971). Glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) activities have been found in Plasmodium knowlesi (Natochin and Seravin, 1962), while only the former enzyme activity has been demonstrated in E. steidae (Frandsen, 1970). In gregarines so far studies of this kind have been made, to the best of our knowledge, only in Gregarina cuneata, Stenophora conjugata and Stylocephalus mesomorphi. Succinate dehydrogenase (SDH) activity has been shown in G. cuneata (Natochin and Seravin, 1962), S. conjugata (Amoji and Rodgi, 1973) and in S. mesomorphi (Amoji, 1975). In addition, activities of NAD linked LDH, SDH, GDH, and MDH have been demonstrated in S. conjugata (Amoji and Rodgi, 1973) and in S. mesomorphi (Amoji, 1975); in the latter species, the activities of G.6-PDH, 6-PF DH, NADH and NADPH diaphorases, α-GPDH, IDH and β-HDH have also been observed (Amoji, 1975). Similarly, phosphorlyase activity has been recorded in the ciliates Tetrahymena pyriformis (Ryley, 1952), Isotricha sp. and Dasytricha sp. (Mould and Thomas, 1958); in flagellates it
has been recorded in *Trichomonas foetus* (Ryley, 1955b; Gompertz and Watkins, 1963), *Euglena gracilis* (Hulbert and Rittenberg, 1962; Goldenberg and Marechal, 1963) and in *Astacia ocellata* (Manners et al., 1964). However, the activity of this enzyme has been observed only in one gregarine species, and that is in *Lecudina tuzetae* (Schrevel and Foquet, 1968).

From the foregoing brief survey of the literature, it is evident that the studies on the respiratory enzymes in gregarines are rather few and confined to the trophic stages only.

In the present work, cytochemical investigations on the presence/absence of phosphorylase and the dehydrogenases of anaerobic glycolysis and tricarboxylic acid (TCA) cycle have been made in the trophozoites, gametes, zygotes and sporozoites of the gregarine *Stylocephalus conoideus*, with a view to find which of the two path-ways, viz., oxidative decarboxylation and anaerobic glycolysis, mentioned above, is operative at a particular stage. The present work also aims at understanding the preferential utilization of carbohydrates, lipids and proteins, during these processes, as evidenced by the activities of the respective dehydrogenases, at different stages.
MATERIALS AND METHODS

Freshly collected host beetles were dissected out and live trophic forms of *Stylocephalus conoides* were obtained. The trophic forms were washed in distilled water and transferred to 'embryo' cups containing different incubating media. Squash preparations of cysts and spores at different stages of development were made on cover glasses and also immediately covered over by the incubating media. The method of Burstone (1962) was followed for the demonstration of succinate dehydrogenase, and Hess *et al.* method (vide Pearse, 1972) has been adopted for the demonstration of isocitrate dehydrogenase, glutamate dehydrogenase, \( \beta \)-hydroxybutyrate dehydrogenase, \( \alpha \)-glycerophosphate dehydrogenase, and lactate dehydrogenase. The substrates and the coenzymes used for the demonstration of the dehydrogenases are shown in Table-5.

In these experiments Nitro blue tetrazolium (MBT) served as an electron acceptor and sodium cyanide (0.1 M) as the respiratory inhibitor. Polyvinylpyrrolidone (PVP) served as an osmotic protector.

The method of Farber and Louviere (1956) modified by Bara *et al.* (1965) was followed for the demonstration of NADH and NADPH diaphorases. The whole specimens or smears
were incubated aerobically at 37° for about 15-20 minutes, fixed in neutral formaline (10%) for about 30 minutes, washed in distilled water and were mounted in glycerol jelly. Specimens/smears incubated in substrate free medium served as controls.

To demonstrate the phosphorylase activity, Takeuchi's phosphorylase-transglycosidase method (vide Pearse, 1961) was adopted. The materials were incubated in the medium consisting of glucose-1-phosphate (Potassium salt) 50 mg., adenosine-5-phosphate 10 mg., glycogen 2 mg., distilled water 15 ml., acetate buffer (0.1M) pH 5.6 10 ml., insulin (20 units/ml.) 1 drop and 5 ml. ethanol, at 37°C aerobically for 2 hours, and treated with 40% alcohol and down-graded to water. After drying in an oven at 37°C, the materials were fixed for 3 minutes in absolute alcohol, and again they were dried in air. Subsequently they were treated with Gram's iodine for 3 minutes and mounted in glycerine. Materials incubated in substrate (glucose-1-phosphate) free medium served as controls.

In order to check the reproducibility of the results, all the above mentioned experiments were repeated five times. Photomicrographs of some important preparations were taken immediately after mounting them in glycerol jelly or glycerine. The results are recorded in Table-6.
Table-5: The Substrates And Coenzymes Used For The Demonstration Of Various Dehydrogenases

<table>
<thead>
<tr>
<th>ENZYMES</th>
<th>SUBSTRATE</th>
<th>CO-ENZYME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Isocitrate dehydrogenase (ICDH)</td>
<td>DL-isocitrate trisodium</td>
<td>NAD</td>
</tr>
<tr>
<td>2. Succinate dehydrogenase (SDH)</td>
<td>Sodium succinate</td>
<td>&quot;</td>
</tr>
<tr>
<td>3. Glutamate dehydrogenase (HDH)</td>
<td>Sodium - L - glutamate</td>
<td>&quot;</td>
</tr>
<tr>
<td>4. B-hydroxybutyrate dehydrogenase (β-HBDH)</td>
<td>DL-β-hydroxybutyric acid sodium salt</td>
<td>&quot;</td>
</tr>
<tr>
<td>5. Lactate dehydrogenase (LDH)</td>
<td>Sodium-DL-lactate</td>
<td>&quot;</td>
</tr>
<tr>
<td>6. α-glycerophosphate dehydrogenase (α-GPDH)</td>
<td>DL-glycerophosphate sodium salt</td>
<td>&quot;</td>
</tr>
<tr>
<td>7. Reduced nicotinamide adenine dinucleotide (NADH) diaphorase</td>
<td>Reduced nicotinamide adenine dinucleotide</td>
<td>&quot;</td>
</tr>
<tr>
<td>8. Reduced nicotinamide adenine dinucleotide phosphate diaphorase</td>
<td>Reduced nicotinamide adenine dinucleotide phosphate</td>
<td>NADP</td>
</tr>
</tbody>
</table>
OBSERVATIONS

Phosphorylase activity

The trophozoites showed a positive reaction in the form of a brownish purple colouration. The colouration was intense in the deutoemerite while it was sparsely scattered in the protomerite (Fig. 68). The colour was of a very short duration and lasted only for 5-10 minutes. Squash preparations of gametes and zygotes yielded negative results. On the contrary, a faint purplish brown colour was seen again in the sporozoites. Owing to rapid disappearance of the colouration within 1-2 minutes, the reaction in the sporozoites could not be photographed. Trophozoites incubated in substrate free medium did not show any colour at all.

Lactate dehydrogenase (LDH)

In the trophozoites the activity of this enzyme was seen in the form of diformazan granules throughout the body (Fig. 69). The granules in the protomerite were larger than those in the deutoemerite. The disposition of the granules in the cortical region as well as in the medullary region was uniform. The reaction was also fairly intense in the gametes, zygotes (Figs. 70 & 71) and it was in traces in sporozoites.
\textbf{\textit{\textalpha}-Glycerophosphate dehydrogenase (\textit{\textalpha}-GPDH)}

The diformazan granules showing the activity of this enzyme were seen throughout the body of the trophozoite (Fig. 72). In the gametes and zygotes however, the intensity of formazan granules was more than that found in the LDH reaction (Figs. 73 & 74). In the sporozoites the formazan granules revealing this enzyme activity were distinct (Fig. 75).

\textbf{Glutamate dehydrogenase (GDH)}

The formazan granules were uniformly distributed (Fig. 76). In the trophozoites these granules were more in the cortical region of the endoplasm than in the central core. In the gametes these granules were small and a few (Fig. 77), while in the zygotes they were large and more in number (Fig. 78). The formazan granules were not found in the sporozoites.

\textbf{\textit{\textbeta}-Hydroxybutyrate dehydrogenase (\textit{\textbeta}-HBDH)}

The diformazan granules indicating the activity of this enzyme were seen throughout the body of the trophozoites (Fig. 79). The granules were concentrated more in the protomerite than in the deutomerite. The granules in the central core were smaller than those in the cortical region.
of the endoplasm. The gametes contained a large number of
distinct diformazan granules (Fig. 80). On the contrary,
there were only 2-3 granules in each zygote (Fig. 81). In
the sporozoites the formazan granules were not found.

Isocitrate dehydrogenase (ICDH) and Succinate
dehydrogenase (SDH):

An intense and uniform distribution of diformazan
granules was found in trophozoites, gametes and zygotest of
S. conoides. The granules were quite large, in fact larger
than the granules found in other enzyme reactions. The
reaction of ICDH was more in the protomerite than in the
deutomerite of the trophozoite (Fig. 82). Gametes and
zygotes also showed a fairly intense reaction. The reaction
in the sporozoites was negative. The activity of SDH in the
trophozoites on the contrary was uniform both in the
protomerite and deutomerite (Fig. 83). There was an intense
activity in the gametes and in zygotes (Figs. 84 & 85). The
sporozoites showed a negative reaction.

Reduced Nicotinamide adenine dinucleotide diaphorase
(NADH-diaphorase) and Nicotinamide adenine dinucleotide
phosphate diaphorase (NADPH-diaphorase):

The localization of reduced nicotinamide adenine
dinucleotide diaphorase (NADH) and nicotinamide dinucleotide
phosphate diaphorase (NADPH) was also observed in the form
of diformazan granules in the trophozoites of *S. conoides*. The diformazan granules were distributed evenly in the protomerite and the deutomerite (Fig. 86). The granules in the cortical region as well as in the central region of the endoplasm were of uniform size. The gametes and zygotes showed 8-10 granules depicting the activity of NADH-diaphorase (Figs. 87 & 88). Similarly the diformazan granules demonstrating NADPH activity were also seen in the gametes and zygotes of *S. conoides*. There was a distinct NADH-diaphorase activity in the sporozoites (Fig. 89) whereas NADPH activity was found in traces (Fig. 90).
DISCUSSION

It is well known that in the catabolism of polysaccharides phosphorylase is invariably involved (Oser, 1965). The occurrence of phosphorylase has been biochemically demonstrated in ciliates *Tetrahymena pyriformis* (Ryley, 1952); *Isotricha* sp. and *Dasytricha* sp. (Mould and Thomas 1958); among the flagellates the enzyme has been demonstrated in *Trichomonas foetus* (Ryley, 1955b); its biochemical variety, namely, uridine diphosphoglucose (UDPG) phosphorylase has been extracted from *Euglena gracilis* (Hurlbert and Rittenberg, 1962) and *Trichomonas foetus* (Gompertz and Watkins, 1963); similarly another biochemical variety, laminoribose phosphorylase has been identified in *Euglena gracilis* (Goldemberg and Marechal, 1963) and *Astacilla ocellata* (Manners et al., 1964). Among the coccidians, phosphorylase enzyme has been demonstrated in the endogenous stages of *Eimeria stiedae* (Frandsen, 1970). This enzyme activity has been demonstrated adopting autoradiographic and cytochemical techniques in the trophozoites of only one species of gregarine, *Lecudina tuzetae* (Schrevel and Fouquet, 1968). In the present work, the enzyme activity has been observed not only in the trophic stages but also in the sporozoites of *S. conoides*. It is interesting to note that the enzyme activity is not found in the gametes.
and zygotes. This absence may be correlated with lack of polysaccharide reserves in these bodies of *S. conoides* (vide Chapter I). Though this enzyme has been observed even in the cephalonts and early trophozoites in which building up of polysaccharide reserves is in active phase (vide Chapter I), it is difficult to conclude that the enzyme is actually concerned with synthesis of polysaccharides. Ryley (1967) observes, "Although phosphorylase has been demonstrated in a few species of protozoa, and in some cases has been shown capable, in vitro, of mediating polysaccharide synthesis as well as break-down, it is not certain that phosphorylase has a synthetic role in the cell." However, the active participation of this enzyme in the catabolism of polysaccharides in various cells has been recognized (Oser, 1965).

**Tricarboxylic acid (TCA) cycle:**

Of the three main respiratory metabolic pathways viz., the oxidative decarboxylation (TCA cycle), the pentose path-way and the anaerobic glycolysis, only the tricarboxylic acid (TCA) cycle serves as the principal means to bring about a complete oxidation of the major metabolites - the carbohydrates, lipids and the proteins (Oser, 1965). The presence of succinate dehydrogenase (SDH) in protozoans and
other organisms has been taken as an evidence of the TCA cycle in operation (Natachin and Seravin 1962, and Ryley 1967).

In recent years different dehydrogenases, isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) have been cytochemically demonstrated in the ciliates, Opalina carolinensis (Hunter, 1955), Stylonychia pustulata (Hunter, 1959a), Balantidium coli (Sharma and Bourne, 1963a), Opalina ranarum (Amoji and Rodga, 1974), and Nyctotherus georgii (Amoji, 1975); in the flagellates, Chilomonas sp. (Natachin and Seravin, 1962), and Trichomonas vaginalis (Sharma and Bourne, 1963a & b; Tanaka, 1970); Entamoeba histolytica among the rhizopods (Kalra et al., 1968); in the sporozoans Eimeria intestinalis (Beier, 1962), Eimeria stiedae (Frandsen, 1968) and Toxoplasma gondii (Akao, 1971). Natachin and Seravin (1962) were the first to demonstrate succinate dehydrogenase (SDH) activity in Gregarina cuneata. Subsequently Amoji and Rodga (1973) demonstrated the activities of ICDH, SDH and MDH in another gregarine Stenophora conjugata, and Amoji (1975) made similar observations in one more species Stylocephalus mesomorphi. In the present work besides observing the activities of these dehydrogenases in the trophozoites we have also observed the activities of these enzymes in the
gametes and zygotes of *Stylocephalus conoides*. Concurrent activities of the two coenzymes, reduced NAD and NADP-diaphorases in all these stages indicate that the breakdown of the metabolites ends in the terminal electron transfer in these stages. Histochemical demonstration of diaphorase activities is an indirect evidence for the existence of cytochrome reductases as well (Pearse, 1972). This in turn indicates the occurrence of the cytochrome system. It is of interest to note that in the gametes of *S. conoides* which are almost devoid of carbohydrates but rich in the lipids (Chapter I), the activity of β-hydroxybutyrate (β-HBDH) dehydrogenase is maximum, whereas in the zygotes which are also poor in the carbohydrate reserves (Chapter I) glutamate dehydrogenase (GDH) activity is the most intense. The observations suggest that the respiration in the gametes is based on lipids; and the zygotes GDH activity may indicate more the synthesis of proteins rather than their utilization in respiration. While dealing with the carbohydrate storage and respiration in trypanosomes, Ryley (1967) observes, "None of the group stores significant amount of polysaccharide, and in the case of those organisms which can sustain an appreciable degree of endogenous metabolism such as *Trypanosoma cruzi* and *Strigomonas oncopelti* (Ryley 1963), respiration seems to be based on fat or protein rather than
carbohydrate." Finally, the negative results for any of the TCA-cycle dehydrogenases, obtained in the sporozoites despite the fact that they do contain the carbohydrate reserves, only suggest that they are probably deprived of the oxygen supply from outside by the spore coat.

Gonn and Stumpf (1969) have emphasized the role glutamate dehydrogenase (GDH) in the nitrogen metabolism of organisms. In the life cycle of gregarines, zygote is a stage wherein a considerable amount of protein synthesis has to take place in order to build up a proteinaceous spore capsule on the outside and eight new cells—the sporozoites within the spore. A high activity of GDH in the zygotes of *S. conoides* may well indicate the role of this enzyme in the protein metabolism.

**Anaerobic glycolysis:**

Under anaerobic conditions, energy metabolism is based on the utilization of carbohydrate; anaerobic glycogen fermentation in the absence of carbon dioxide results in the production of lactic acid (Ryley, 1967). Cytochemical activities of α-GPDH and LDH are regarded as the indicators of that part of glycolytic system in which, under anaerobic condition, glucose is consumed and glycerol and lactate are
produced respectively (Bearse, 1961). During the process these two dehydrogenases also play an important role in regeneration of the coenzyme A (NAD) without which the very glycolytic process cannot proceed uninterruptedly (Conn and Stumpf, 1969).

Levy and Scherbaum (1965) observed anaerolic glycolysis in the freshwater ciliate *Tetrahymena* sp. during night time. Vickerman (1965) observed the presence of only glycolytic enzymes and the absence of the Krebs cycle enzymes in the cell-free extractions of blood stream forms of *Trypanosoma* sp. Frandsen (1970) has reported the presence of lactate dehydrogenase (LDH) in sporozoites of *Eimeria steidae*. In recent years cytochemical demonstration of both LDH and α-GPDH activities have been shown in the ciliates *Nyctotherus georgei* and *Opalina ranarum* (Amoji and Rodgi, 1974), in trophozoites of gregarines *Stenophora conjugate* (Amoji and Rodgi, 1973) and *Stylocephalus mesomorphi* (Amoji, 1975). In both these gregarines, the activity of α-GPDH is more than that of LDH. In our observations on *S. concoides*, a sharp distinction between the intensities of the activities of these two dehydrogenases was not found in the trophozoites; however, in the gametes and zygotes α-GPDH activity was more than LDH activity. In sporozoites only α-GPDH activity was observed whereas LDH activity was found in traces or
was absent. It is therefore inferred that in these stages and also to some extent in the trophozoites lactate production is less than the production of glycerol. Chefurka (1965; cited in Amoji, 1975) has found a correlation between the amounts of the end products of glycolysis and the relative activities of the two enzymes. In this way it is clear that in the glycolytic pathway in *S. conoides* α-GPDH plays a more important role than LDH. It is interesting to note here that in the sporozoites unlike in other stages of *S. conoides*, only LDH and α-GPDH activities, the latter in particular, were observed. This is an indication that only anaerobic glycolysis takes place in the sporozoites. However, it is emphasized that this is just a suggestion which needs confirmation, particularly measurement of oxygen consumption by the spores.

Our demonstration of different respiratory dehydrogenases in different stages of *S. conoides* reveals that all the stages except the sporozoites, have the potential to adopt either the aerobic respiration or anaerobic glycolysis, if needed, while the sporozoites have to depend only on anaerobic glycolysis. Such a respiratory switch from one type to another has been reported in *Trypanosoma* spp., during their blood cell phase and mosquito midgut phases (Vickerman, 1965). In *Tetrahymena* sp.
Levy and Scherbaum (1965) observed oxidative metabolism during the daytime and anaerobic glycolysis during the night.

**NADH and NADPH diaphorases:**

Diaphorases are the enzymes catalyzing the oxidation of reduced coenzymes, NADH and NADPH, the oxidation being brought about by different electron acceptors such as flavoproteins and cytochromes. Histochemically the diaphorase activity can be shown using the tetrazolium salts in the incubating medium (Pearse, 1972).

Histochemically the diaphorases have been localized only in one flagellate *Trichomonas vaginalis* (Sharma and Bourne, 1964d), in the ciliates *Opalina ranarum* (Amoöji and Rodig, 1974) *Nyctotherus georgii* (Amoöji, 1975) and in the gregarines *Stenophora conjugata* and *Stylocephalus mesomorphus* (Amoöji, 1975) and in different stages of *S. conoides*, in the present work.

As the reoxidation of the reduced NAD and NADP is essential for the continuous operation of the TCA cycle and also the anaerobic glycolysis, it is obligatory that every organism must be provided with a means to rebuild the NAD and NADP from their reduced forms. Further, in histochemically
demonstrating the level of diaphorase activity the level of cytochrome reductase is also demonstrated (Pearse, 1972). From this observation, it may be inferred that in gregarines the cytochrome system, concerned with the terminal electron transfer, is in existence. Finally, in this context, it may be pointed out that in sporozoites of S. conoides, in which only anaerobic glycolysis operates, NAD diaphorase alone is present and NADP diaphorase is in traces.

From the foregoing account, it is evident that in the gregarine S. conoides, the trophic forms, the gametes and zygotes have the potentiality to adopt anaerobic glycolysis and also the oxidative decarboxylation of carbohydrates, lipids and proteins, whereas the sporozoites have the ability to bring about the degradation of carbohydrates only through anaerobic glycolysis.
The presence of phosphorylase, \( \beta \)-hydroxybutyrate dehydrogenase (\( \beta \)-HEDH) and glutamate dehydrogenase (GDH) have been cytochemically demonstrated in the trophozoites of the gregarine \textit{Stylocephalus conoides}. Phosphorylase activity was also seen in the sporozoites but not in the gametes and zygotes; the activities of \( \beta \)-HEDH and GDH were seen in the gametes and zygotes and not in the sporozoites.

Activities of succinate and isocitrate dehydrogenases (NAD dependent) of the tricarboxylic acid (TCA) cycle and lactate and \( \alpha \)-glycerophosphate dehydrogenases of anaerobic glycolysis have been cytochemically demonstrated in the trophozoites, gametes and zygotes of \textit{S. conoides}. In the sporozoites, however, activities of SDH and ICDH of the TCA-cycle were not observed; on the contrary, only LDH and \( \alpha \)-GPDH activities were observed.

Cytochemical demonstration of reduced NAD and NADP diaphorase activities has also been carried out in the trophozoites, gametes and zygotes. In the sporozoites, however, only reduced NAD diaphorase activity was conspicuous.
4. On the basis of our findings, it is suggested here that in the sporozoites, the enzyme system of only the anaerobic glycolysis is present, while in the remaining stages the enzyme systems of oxidative decarboxylation of carbohydrates, lipids and proteins and also anaerobic glycolysis are present.

5. A respiratory shift from the aerobic type to anaerobic type has been observed in the life-cycle of the gregarine during the spore-stage.
Table-6: Respiratory Enzymes In Different Stages In The Life-Cycle Of The Gregarine *Stylocephalus conoides* Devdhar.

<table>
<thead>
<tr>
<th>No.</th>
<th>Respiratory Enzymes</th>
<th>Trophozoite</th>
<th>Protomerite</th>
<th>Deutomerite</th>
<th>Gametes</th>
<th>Zygotes</th>
<th>Sporozoites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Phosphorylase</td>
<td>+</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Lactate dehydrogenase (LDH)</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>α-Glycerophosphate dehydrogenase (α-GPDH)</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Isocitrate dehydrogenase (ICDH)</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Succinate dehydrogenase (SDH)</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Glutamate dehydrogenase (GDH)</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>β-Hydroxybutyrate dehydrogenase (β-HBDH)</td>
<td>++++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Reduced Nicotinamide adenine dinucleotide (NADH) diaphorase</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Reduced Nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

++++ = Maximum activity; + = Minimum activity; 
+ = Traces of activity; - = No activity