

**LOCALIZATION OF SOME CYTOCHEMICAL SUBSTANCES IN *RAMICEPHALUS OLIVACUS* SARKAR & HALDAR (PROTOZOA, APICOMPLEXA: EUGREGARINIDA) OF THE DRAGONFLY *CERIAGRION OLIVACEUM* LAIDLAW (ZYGOPTERA: COENAGRIONIDAE)**

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**Abstract** — The cytochemical study of the various life history stages of the cephaline gregarine *R. olivacus*, obtained from the odonate *C. olivaceum*, reveals a very strong reaction of PAS positive substances in the endoplasm (except the nucleus) of all the life history stages while the nuclear substances react negatively to the Feulgen test except the nuclei of the sporozoites which show moderate to strong reaction for DNA. The simple lipid has been demonstrated weakly in the pellicle, the nuclear membrane and also in the endoplasm of the sporadin in scattered form. The general protein granules have been localized in the pellicle, the endoplasm as well as in the nuclei of the trophozoite and the sporadin. The granules in and around the nuclei of the same show a stronger reaction with Mercury Bromophenol Blue. The role played by these substances has been discussed in the light of the bionomics of the gregarine.

**Introduction**

The only cytochemical study of an odonate gregarine is that of STEIN (1960) and this encourages us to study the localization of some cytochemical substances in the various life history stages of the odonate gregarine *Ramicephalus olivacus*.

**Methods**

Smears of the midgut content of live *Ceriagrion olivaceum* infected by the gregarine *Ramicephalus olivacus*, were made on clean glass slides. Some were fixed in Carnoy's fluid and were subjected to the Mercury Bromophenol Blue method for general protein, the PAS technique for general polysaccharides and the Feulgen test for DNA. A few smears were fixed in 10.0% neutral Formaline for simple lipid. All the methods were followed after PEARSE (1968). The controls were maintained where necessary.

The figures were drawn with the aid of a camera lucida.

**Results**

**Mercury Bromophenol Blue test** — The pellicle and the endoplasm show some uniformly distributed positive granules while the granules in and around the nucleus indicate stronger reaction in the trophozoite and the sporadin (Figs 1-2).

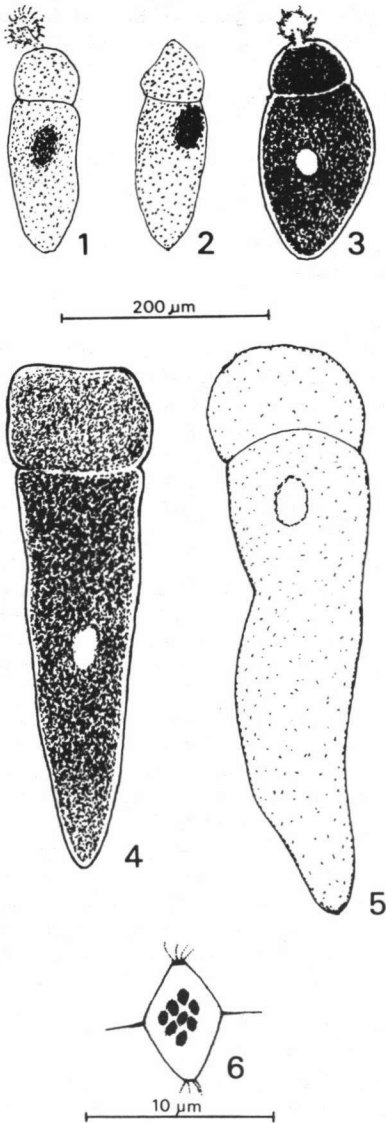
**PAS test** — Although the PAS positive substances have been found in the endoplasm of both protomerite and deutomerite of the trophozoite and the sporadin as large violet granules; the pellicle, ectoplasm, epimerite and the nuclei of both trophozoite and sporadin show a completely reverse negative reaction (Figs 3-4).

**Sudan Black B test** — Black Sudan Black B positive granules (simple lipid) have been observed in the pellicle, endoplasm and the nuclear membrane of the sporadin with a reaction of moderate intensity (Fig. 5).

**Feulgen test** — Feulgen positive substances have not been found either in the cytoplasm or in the nucleus of the trophozoite or sporadin. However, a moderate to strong positive reaction was found in the nuclei of the sporozoites housed in the sporocyst (Fig. 6).

**Discussion**

The nuclei of the trophozoite and the sporadin did not respond to the Feulgen test. However, the nuclei of the sporozoites reacted strongly indicating a high level of DNA. This phenomenon was also found by STEIN (1960). The DNA content of the nuclei at the trophozoite and sporadin stages might be below the sensitivity level of the Feulgen test (BHAT-TACHARYA & CHAKRAVARTY, 1976). This low DNA content possibly accounts for the fact that the parasite at these stages does not



Figs 1-6. Localization of various cytochemical substances at different stages in the life cycle of *Ramicephalus olivacus*: (1-2) Distribution of Mercury Bromophenol Blue positive substances in the trophozoite and sporadin, respectively; — (3-4) Distribution of PAS positive substances in the trophozoite and sporadin respectively; — (5) Distribution of Sudanophilic B substances in the sporadin; — (6) Distribution of Feulgen positive substances in the sporozoites nuclei of the sporocyst.

depend on its own system. On the contrary, the host's system indirectly supports the metabolic activity of the parasite. However, the high level of DNA of the sporozoite nucleus might explain the fact that the sporozoites might have developed their own systems for further invasion into a new host and passes to the trophozoite stage.

The very high glycogen content of the trophozoite, especially of the sporadins or gamonts, might function as a food reserve since the parasite after those stages goes through an independent phase as well as a phase outside the host until it invades a new host as sporozoite stage.

Protein, seen in the trophozoite and sporadin as blue granules, is moderate in reaction and its granules are distributed uniformly. The same intensity could not be observed in the sporozoites of the sporocyst. Probably, protein plays an important role in the formation of the gametocyst and sporocyst wall (BHATTACHARYA & CHAKRAVARTY, 1976).

Black granules of simple lipid were found in moderate concentration in the sporadin or gamont stage. The simultaneous presence of glycogen and simple lipid prior to the sexual phase might be regarded as the preparation for the complex and independent metabolic processes during the gametocyst and sporocyst stages in the life cycle of the odonate gregarine.

Till now, the nature of the relationship between the gregarine and its odonate host is not very clearly understood (ABRO, 1971, 1974; SARKAR, 1981). However, a thorough study on this subject, especially at the biochemical level, might answer the question in future.

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