CHAPTER III

Histochemical demonstration of some hydroxysteroid dehydrogenases in the gregarines
Introduction:-

The biosynthesis of steroids including the sex-steroids and their in vitro conversion have been studied in a number of metazoan invertebrates, viz. in nematodes (Brigs, 1972), in insects (Lehoux & Sandor, 1969 and 1970; Dube & Lemonde, 1970; Sandor et al., 1975; Trandabura and Tasca, 1976; Sandor and Medhi, 1979; Hurkadali, 1982; Declerck et al., 1983 and 1984; Ohnishi et al., 1985; Ogiso et al., 1986), in molluscs (Gottfried and Dorfman, 1970a, 1970b, and 1970c; DeLongcamp et al., 1974, Carreau and Dorsadowsky, 1974 and 1977 and Gouder et al., 1977) and in echinoderms (Hathway, 1965; Schoenmakers 1979 and Schoenmakers and Voogt, 1980). The pathway involved in the process are shown to be similar to those operating in the vertebrates (Barrington, 1972 and Sandor and Medhi, 1979).

Likewise, biosynthesis of steroids and sterols have also been reported in a few protozoans other than sporozoans (Sebeck & Michaels, 1957; Conner et al., 1966 and reviews; Wagtendonk, 1955; Wright, 1964; Shorb, 1964; Charney and Herzog, 1967; Dewey, 1967 and Von-brand, 1973). On the otherhand, Hutner (1964), believes that the protozoans whether free-living or parasitic, depend upon the exogenous supply of steroids. Gutteridge and Coombs (1977), opine that most of the parasitic protozoa are incapable of steroid biosynthesis and hence must procure them from the ambient medium.

Clark and Boch (1959b) and Norris et al. (1969) state that, the symbiotic microorganisms which some insects harbour may synthesize the cholesterol, the precursor of steroids. Recently in
sporozoea the cholesterol has been reported in two species of gregarines, viz. *Stylocephalus mesomorphi* (Amoji, 1975) and *Stylocephalus conoides* (Desai and Nadkarni, 1987). Further, the occurrence of some key steroid dehydrogenases have also been demonstrated by cytochemical methods in *S. conoides* (Desai and Nadkarni, 1987) indicating the steroidogenic potential of the gregarine.

In the present investigation, an attempt has been made to present cytochemical evidence for the presence of some key steroid dehydrogenases such as \( \Delta^5-3\beta\)-hydroxysteroid dehydrogenase (\( \Delta^5\beta\)-HSDH) and \( 17\beta\)-hydroxy-steroid dehydrogenase (\( 17\beta\)-HSDH) and Glucose-6-phosphate dehydrogenase (G-6-PDH) involved in the synthesis/metabolism of steroids including the sex-steroids in some more gregarine species and find out whether any clue can be obtained in understanding the sexual dimorphism in these organisms.

**Materials and Methods:**

Syzygies of trophozoites/gamonts of *Gregarina cuneata* were obtained from the freshly collected *Tenebrio molitor* larvae. The syzygies of trophozoites/gamonts of *Hirmocystis speculitermis* and *Steinina termitis*, were obtained from the freshly collected termite workers of *Speculitermes cyclops sinhalesis*, while those of *Hirmocystis incola* were obtained from the termite workers of *Capritermes incola*.

**Demonstration of steroid dehydrogenases:**

A) \( \Delta^5-3\beta\)-Hydroxysteroid dehydrogenase (\( \Delta^5\beta\)-HSDH) and \( 17\beta\)-hydroxy-steroid dehydrogenase (\( 17\beta\)-HSDH)

The trophic forms were taken on the slide, cleaned with
distilled water and frozen over dry ice vapours at about-50°C for 5 minutes and immediately thawed at room temperature. After a brief rinsing in cold acetone (4°C), the preparations were incubated in appropriate media at 37°C under aerobic conditions for half an hour to three and half hours for different dehydrogenases. These preparations were fixed in 10% neutral formalin for 30 minutes and washed in distilled water and mounted in glycerol jelly. For the demonstration of hydroxysteroid dehydrogenases, Wattenberg's method (1958) modified by Levy et al. (1959) and subsequently modified by Baillie et al. (1966) was adopted.

The incubation medium consisted of the steroid substrate 1 mg/ml dissolved in dimethyl formamide (DMF), the co-enzyme B-nicotinamide adenine dinucleotide (B-NAD) 1.5 mg/ml, nitro-blue tetrazolium (NBT) salt 1 mg/ml in 0.2M phosphate buffer (pH 7.4).

The following specific steroid substrates were used for the cytochemical demonstration of (a) $\Delta^5$-3β-hydroxysteroid dehydrogenase ($\Delta^5$-3β-HSDH) and (b) 17β-hydroxysteroid dehydrogenase (17β-HSDH) activities.

a) $\Delta^5$-3β-HSDH activity

1. Dehydroepiandrosterone (DHEA) (3β-hydroxyandrost-5-ene-17-one)
2. Pregnenolone (3β-hydroxyprogren-5-20-one)
b) 17B-HSDH activity

1. 17B-estradiol
   (3, 17\beta-dihydroxy-1,3,5, (10)-estratriene).
2. Testosterone
   (17B-hydroxyandrost-4-en-3-one).

Some trophozoites/gamonts were incubated in the substrate-free medium under identical conditions served as controls for the hydroxysteroid dehydrogenase activity in general. A second sample of trophozoites was treated for 10 minutes with Cyanoketone dissolved in a few drops of 0.2M phosphate buffer (pH 7.4) and was then incubated in the normal medium containing the substrate dehydroepiandrosterone (DHEA). This sample served as the control specifically for $\Delta^5-\beta$-hydroxysteroid dehydrogenase activity.

C) Glucose-6-phosphate dehydrogenase (G-6-PDH):

For the demonstration of glucose-6-phosphate dehydrogenase activity, the method of Cohen (1959) modified by Saidapur and Nadkarni (1972), was adopted. Trophozoites were incubated in a medium consisting of D-glucose-6-phosphate disodium salt (1.5 mg/ml), the co-enzyme nicotinamide adenine dinucleotide phosphate (NADP) (0.5 mg/ml) and nitroblue tetrazolium salt (NBT) (0.5 mg/ml) dissolved in 0.2M phosphate buffer (pH 7.2). Samples of trophozoites incubated in the substrate-free medium served as controls.

The intensities of the enzyme activity as indicated by the deposition of diformazan granules were visually graded as follows:
(-) = No activity
(+±) = Traces of activity
+ = Minimum activity
++++ = Intense activity

The trophozoites in syzygies showing more 17β-HSDH activity with testosterone as substrate have been interpreted as males, as this dehydrogenase is involved in the interconversion of testosterone androstenedione and the trophozoites showing more 17β-HSDH activity with 17β-estradiol substrate as the females as this isomer is involved in the interconversions of estradiol-17β-estrone.

All the chemicals used in the present work are of 'Sigma Grade' obtained from the Sigma Chemical Company, U.S.A. The results are shown in Table IX and are supported by some important microphotographs.

Observations
A) Steroid dehydrogenases
a) \( \Delta^5 \)-3β-Hydroxysteroid dehydrogenase (\( \Delta^5 \)-3β-HSDH)

After incubating the smear preparations for 90 minutes the formation of the blue diformazan granules indicating the presence of \( \Delta^5 \)-3β-HSDH developed throughout the body of trophozoites and gamonts of all the four species, viz. Gregarina cuneata, Hirmocystis specularis, Hirmocystis incola and Steinna termitis. (Fig.23, 24, 25 and 26). The granules were more concentrated around the
nuclear wall. The trophozoites incubated in the substrate-free medium, showed a few or no diformazan granules (Fig. 27). In cyanoketone treated trophozoites, no diformazan granules were observed (Fig. 28).

No difference in the intensity of $\Delta^5$-3$\beta$-HSDH activity could be seen in the primates and satellites of all the four species. However, $\Delta^3$-3$\beta$-HSDH activity obtained in these gregarines with dehydroepiandrosterone (DHEA) as the substrate, the formation of the diformazan granules was more than that with progrenolone as the substrate, indicating the preferential utilization of DHEA by these gregarines.

b) 17$\beta$-Hydroxysteroid dehydrogenase (17$\beta$-HSDH) activity

Blue diformazan granules developed in the trophozoites and gamonts of the above said species, indicating the 17$\beta$-hydroxy-steroid dehydrogenase (17$\beta$-HSDH) activity but the required incubation period was as long as 3½ hours. The granules just beneath the ectoplasm were larger than those found deep in the endoplasm. In the endoplasm they were more concentrated around the nucleus than elsewhere in all the four species (Fig. 29, 30, 31, 32, 35, 36, 37 & 38).

The satellites of G. cuneata and H. speculitermis and the primates of H. incola and S. termitis showed the preference to the substrate 17$\beta$-estradiol (Figs. 29, 30, 31 & 32) while the primates of G. cuneata and H. speculitermis and the satellites of H. incola and S. termitis showed the preference to the substrate testosterone (Figs. 35, 36, 37 & 38) as indicated by the more concentration of
diformazan granules. Controls, either did not show the enzyme activity or showed very weak activity in all the four species of gregarines (Figs. 33, 34, 39).

B) Glucose-6-Phosphate-dehydrogenase (G-6-PDH) activity:

The Glucose-6-phosphate-dehydrogenase (G-6-PDH) activity was seen throughout the trophozoites and gamonts of all the four species (Figs. 40, 41, 42 & 43). The diformazan granules were large but were less dense, in the protomerite, whereas they were small but densely distributed in the deutomerite. The distribution of these granules was uniform in the primaries and satellites. In control preparations, few diformazan granules were observed (fig. 44 & 45). The incubation period required was only 20 to 30 minutes.

The intensity of the activity of the afore-said enzymes varied. G-6-PDH yielded a more intense activity than the other two enzymes. Of the two substrates used for demonstration of \( \Delta^5-3\beta\)-HSDH, the substrate DHEA yielded a more intense activity than pregnenolone. The activity obtained for \( 17\beta\)-HSDH was less intense than that obtained for \( 13\beta\)-HSDH.

Discussion:

Barrington (1968) states that steroid biosynthesis is not an exclusive prerogative of vertebrates, since the steroid biosynthesis, is widespread throughout the animal kingdom. The occurrence of steroids and steroid dehydrogenases has been reviewed by Sandor et al. (1975). The occurrence of steroids and sterols in different species of protozoa, free-living as well as
parasitic has been reviewed by Wagtendonk (1955), Wright (1964), Shorb (1964), Dewey (1967) and Von Brand (1973).

The protozoan species studied are *Entamoeba coli*, *Amoeba* sp., *Euglena* sp., *Trichomonas* sp. and *Paramecium aurelia*. The sterols and steroids extracted from these organisms are cholesterol, stigmasterol, $\beta$-stigmasterol, glucocorticoids, testosterone and progesterone. Eventhough some representative species belonging to *Sarcodina*, *Mastigophora* and *Ciliophora* have been studied by earlier workers (Sebeck and Michaels, 1975; Wright, 1964; Conner et al., 1966) Sporozoan representatives have not been examined earlier. The symbiotic microorganisms which some insects harbour may synthesize cholesterol, the precursor of steroids (Clark and Bloch, 1959b and Norris et al., 1969). Only recently, presence of cholesterol has been observed in two gregarine species viz. *Stylocephalus mesomorphi* (Amoji, 1975) and *Stylocephalus conoides* (Desai and Nadkarni, 1987). In addition, in the latter species the presence of certain key steroid dehydrogenases has been demonstrated by cytochemical methods (Desai and Nadkarni, 1987).

Sebeck and Michaels (1957). have demonstrated the in vitro conversion of steroids in *Trichomonas vaginalis* and *Euglena gracilis* in a manner similar to those in mammalian tissues and have shown that they contain steroid dehydrogenase which specifically converts 17-keto and 17$\beta$-hydroxyl groups of certain C$_{18}$ and C$_{19}$ steroids. Wright (1964) has shown that *Entamoeba coli* has the ability to incorporate radioactive stigmasterol, which suggests
its steroidogenic potential. Further, Wright (1964) has observed in Amoeba sp. the aggregatory stimulus provided by a steroid acrasin (\(\Delta^{22}\)-Stigmastenol-3B-ol) and also by the sex steroids, estradiol, progesterone and testosterone. Conner et al. (1966) have shown that the ciliate Tetrahymena pyriformis has the ability to convert cholesterol into cholesta-5,7,22 triene, 3B-ol. Charney and Herzog (1967) have studied the biosynthesis of testosterone in Euglena gracilis and testosterone and estrone in Trichomonas sp. On the other hand, the anaerobically grown yeasts, some parasitic Trichomonads, Paramecium sp. and Tetrahymena sp. are known to depend upon the exogenous supply of steroids (Hutner, 1964). Gutteridge and Coombs (1977) opine that parasitic protozoa procure the steroids from the ambient medium.

In vertebrate tissues, some structural aspects of cells are often correlated with steroid synthesis. It is reported that steroidogenic cells in the gonads of vertebrates are characterised by the presence of smooth endoplasmic reticulum and spherical mitochondria with tubular cristae (Christensen and Gillum, 1969). Similar mitochondria have been described in Pelomyxa carolinensis (Reuben, 1955), Amoeba proteus (Grieder et al., 1958), Mastigophora locustae (Harry and Finlayson, 1976) and in the gregarine Didymophyes gigantea (Hildbrand, 1976). In the schizonts and gametocytes of Eimeria tenella, the occurrence of both rough and smooth endoplasmic reticulum and numerous mitochondria with few cristae is reported (McLaren, 1969). Both rough and smooth endoplasmic
The presence of free fatty acids, cholesterol and the activity of G-6-PDH and NADH diaphorase in the trophozoites of S. mesomorphi has been reported (Amoji, 1975). Occurrence of rich neutral lipids, cholesterol and certain key enzymes involved in the biosynthesis of steroids viz. \( \Delta^5 \)-3\( \beta \)-HSDH, 11\( \beta \)-HSDH and 17\( \beta \)-HSDH in another gregarine species, viz. S. conoides indicate the organism's steroidogenic potentials (Desai and Nadkarni, 1987).

Rich neutral lipids and the activity of steroid dehydrogenases such as \( \Delta^5 \)-3\( \beta \)-HSDH and 17\( \beta \)-HSDH are observed in the trophozoites/gamonts of the four species of gregarines viz. G. cuneati, H. speculitermis, S. termitis and H. incola in the present study. The presence of \( \Delta^5 \)-3\( \beta \)-HSDH and 17\( \beta \)-HSDH suggests their potential to synthesise metabolise the steroids including the sex-steroids. The pathways involved in the synthesis/metabolism of steroids in invertebrates are shown to be similar to those operating in the vertebrate tissues (Barrington, 1972; Sandor and Medhi, 1979).

It is well known that the biosynthesis of the hormonally active steroids in the steroid-producing tissues involves the conversion of \( \Delta^5 \)-3\( \beta \)-hydroxysteroids to \( \Delta^4 \)-ketosteroids and the enzyme system carrying out this transformation is shown to be \( \Delta^5 \)-3\( \beta \)-hydroxysteroid dehydrogenase (\( \Delta^5 \)-3\( \beta \)-HSDH) & its presence has been...
histochemically demonstrated in all the steroidogenic tissues of vertebrates (Samuels, et al., 1951, Von Oordt, 1963, Dorfman and Unger, 1965, Baillie, et al., 1966, and Rubin et al., 1969). The cytochemical demonstration of Δ⁵-3β-HSDH activity in cells permits one to infer that they actively participate in the biosynthesis/metabolism of steroids (Van Oordt, 1963). The histochemical demonstration of Δ⁵-3β-HSDH activity is generally considered as an evidence, albeit indirect, of the steroidogenic/steroid metabolic potential of any organism or tissue under consideration (Lehoux and Sandor, 1970). In the vertebrate biosynthetic scheme Δ⁵-3β-HSDH and Δ⁵-Δ⁴-isomerase effect the transformation of pregnenolone into a host of biologically active C₂₁, C₁₉, and C₁₈ compounds (Lehoux and Sandor, 1970). Δ⁵-3β-HSDH catalyses the conversion of pregnenolone to progesterone, 17-OH pregnenolone to 17-OH-progesterone and dehydroepiandrosterone (DHEA) to androstenedione (Baillie, et al., 1966). Δ⁵-3β-HSDH activity has been demonstrated in a few species of invertebrates, viz. in Schistosomes (Brigs, 1972) in a variety of tissues of the oyster Crassostrea gigas, (Mori et al., 1964), the male phase of ovotestis of V. Templtoni (Gouder et al., 1977), in the spermatids and spermatozoa of two heteropteran insects Graphosoma italicum and Eurydema ventralis (Trandabura & Tasca, 1976), and in the gonads of the lepidopteran Philosamia ricini, Antitheraea mylitta and Bombyx mori (Hurkadi, 1982).

To the best of our knowledge, among the protozoans only in Stylocephalus conoides Δ⁵-3β-HSDH is cytochemically demonstrated so far (Desai and Nadkarni, 1987). Our findings described
in the present chapter suggest that, the gregarines *G. cuneata*, *H. speculitermis*, *S. termitis* and *H. incola* have the enzymes necessary to convert pregnenolone to progesterone and DHEA to androstenedione and thereby their ability to synthesize/metabolise the steroids.

Glucose-phosphate dehydrogenase (G-6-PDH) is involved in generating the reduced nicotinamide adenine dinucleotide phosphate (NADPH) which is required for hydroxylations of steroids during active steroidogenesis (White et al., 1959; Savard et al., 1963; McKerns, 1968 and Weist and Kidwell, 1969). Further G-6-PDH is implicated along with Δ⁵-3β-HSDH in enzymatic transformations related to steroidogenesis in vertebrates (Weist and Kidwell, 1969).

G-6-PDH activity has been demonstrated in the ciliate *Balantidium coli* (Sharma & Bourne, 1964); in *Nyctotherus georgen*, *Opalima ranarum* and in the gregarines *Stenophora conjugata* and *Stylocephalus mesomorphi* (Amoji, 1975), in *S. conoides* (Desai & Nadkarni, 1987) and in the coccidian *Eimeria stiedae* (Frandsen, 1970). Further, G-6-PDH activity has been biochemically isolated from *Eimeria stiedae* (Frandsen, 1975a, b and 1976 and Frandsen and Ennis, 1974).

The activity of G-6-PDH has been demonstrated by histochemical methods in the present work in the trophozoites/gamonts of all the four species of gregarines *G. cuneata*, *H. speculitermis*, *S. termitis* and *H. incola*. Our findings provide an additional support to steroidogenic potential of these sporozoans.

17β-hydroxysteroid dehydrogenase (17β-HSDH) which
plays an important role in the biosynthesis of sex-steroids, the androgens and estrogens. This enzyme catalyses the interconversions of testosterone to androstenedione and vice-versa and 17β-estradiol to estrone and vice-versa in the gonads of the vertebrates (Baillie et al., 1966 and Rubin et al., 1969). The biochemical investigations have shown the occurrence of 17β-HSDH in the gonads of insects (Lehoux and Sandor, 1969 and 1970 and Dube and Lemonde, 1970). The occurrence of 17β-HSDH has been histochemically demonstrated in the spermatids and spermatozoa of the heteropteran insects G. italicum and E. ventralis (Trandabura and Tasca, 1976); and in the gonads of lepidopteran P. ricini, A. mylitta and B. mori (Hurkadli, 1982).

Based on the biochemical studies, Lehoux and Sandor (1970) have inferred that the gonads of insect Gryllus domesticus have two substrate specific 17β-hydroxysteroid dehydrogenases, i.e., testosterone-17β-hydroxysteroid dehydrogenase and estradiol-17β-hydroxysteroid dehydrogenase; and that the testosterone dehydrogenase is different from the estradiol dehydrogenase. When the gonadal homogenate was incubated in the presence of both testosterone and 17β-estradiol, the yield of the 17β-ketosteroid produced were the same as that in experiments, where only one of the substrate was incubated. From this they have inferred that there does not seem to be any competition for the active sites between the androgenic and estrogenic precursors. Further the significant difference in in vitro conversion of testosterone (2%) and 17β-estradiol (50%) has lead to the inference that the gonads of the mussel Mytilus edulis have two 17β-HSDH enzyme
entities (De Longcamp, 1974). The *in vitro* conversion studies on the sperm preparations of oyster (Hathway, 1965) and the gonads of *Mytilus edulis* (De Longcamp et al., 1974) and *Sepia officinalis* (Cageau and Drosdowsky, 1974 and 1977) suggest the occurrence of 17β-HSDH in molluscs. The occurrence of 17β-HSDH has been histochemically demonstrated in kidney and digestive tract of *Crassostrea gigas* (Mori et al., 1965) and in the male-phase ovotestis of *V. templitoni* (Gouder et al., 1977). Similarly *in vitro* studies on the sperm preparations of urchins and on the ovaries of *Asterias rubens* suggest the occurrence of 17β-HSDH activity in echinoderms (Hathway, 1965). Recently, 17β-HSDH activity has been shown by cytochemical methods in the gregarine *Stylocephalus conoides* (Desai and Nadkarni, 1987). Our findings of this enzyme in *G. cunea*, *H. speculitermis*, *H. incola* and *S. termitis* corroborate the findings in *S. conoides*.

De Clerck et al. (1983 and 1984) have identified progesterone, testosterone and several related androgens in larval haemolymph of *Sarcophaga bullata*. Ohnishi et al., (1985) have identified estradiol-17β from the silkworm ovaries. Ogiso et al., (1986) have observed different metabolic activities in the gonads of *B. mori*, i.e., the ovaries of *B. mori* converted estrone to estradiol-17β and vice-versa but testosterone was not appreciably changed. Further, the testes converted the testosterone to several metabolites but estradiol was not converted like-wise.

Gottfried (1970a, 1970b & 1970c) have shown in the mollusc *Anolimax californicus* dehydroepiandosterone...
and 11-ketosterone can block the inhibitory activity of the right optic tentacle on spermatogenesis. Mori et al. (1965) have shown that the treatment with 17β-estradiol accelerates the sexual maturation in female C. gigas and they have also shown the sex-reversal from male to female in this species after 17β-estradiol administration. These studies indicate some role of steroid hormones in the regulation of sexual reproduction in invertebrates.

It might be recalled here that Wright (1964) has observed the aggregatory stimulus by a steroid acrasin (Δ22-stigmasterol-3β-ol) and also by the sex steroids, estradiol, progesterone and testosterone in Amoeba sp. Charney and Herzog (1967) have studied the biosynthesis of testosterone in Euglena gracilis and testosterone and estrone in Trichomonas sp.

In the present investigation, 17β-HSDH activity is cytochemically demonstrated in the trophozoites/gamonts in the syzygies of four species of gregarines using both the substrates testosterone and estradiol 17β, which suggests the presence of both the stereospecific 17β-HSDH in these gregarines, thereby suggesting the potential of these gregarines to synthesize/metabolise the sex steroids comparable to higher invertebrate and vertebrate gonads. The preferential utilization of the two substrates also suggests that these gregarines may have two separate specific entities of 17β-hydroxysteroid dehydrogenases, namely, testosterone 17β-hydroxy-steroid dehydrogenase and estradiol 17β-hydroxysteroid dehydrogenase.
The preferential utilization of testosterone substrate by the primites of *G. cuneata* and *H. speculitermis* and the satellites of *H. incola* and *S. termitis* suggests that they might be sex-wise the 'males' as this enzyme catalyses the interconversions of testosterone to androstenedione (androgens). And conversely preferential utilization of 17β-estradiol substrate by the satellites of *G. cuneata* and *H. speculitermis* and the primites of *H. incola* and *S. termitis* suggests that they might be sex-wise the females as this enzyme catalyses the interconversions of 17β-estradiol to estrone (estrogen).
SUMMARY

1. The presence of $\Delta^5$-3\textbeta-hydroxysteroid dehydrogenase (\$\Delta^5$-3\textbeta-HSDH) and 17\textbeta-hydroxysteroid dehydrogenase (17\textbeta-HSDH) is histochemically demonstrated in the four species of gregarnes viz. G. cuneata, H. speculitermis, H. incola and S. termitis.

2. The occurrence of $\Delta^5$-3\textbeta-HSDH in all the four species of gregarnes indicates their potential to synthesize/ metabolise the steroids.

3. The occurrence of 17\textbeta-HSDH with both the substrates i.e., testosterone and estradiol 17\textbeta, suggests the potential of these gregarnes to synthesize/metabolise the sex steroids in a manner similar to higher invertebrate and vertebrate gonads.

4. The preferential utilisation of either the testosterone or estradiol 17\textbeta, indicates that they might be having two stereospecific 17\textbeta-hydroxysteroid dehydrogenases. The trophozoites/gamonts showing the preferential utilisation of estradiol 17\textbeta-substrate, are suggested to be the 'females' and those showing the preferential utilisation of 'testosterone' substrate are suggested to be the 'males'. 
The occurrence of Glucose-6-phosphate dehydrogenase (G-6-PDH) activity in these provides an additional evidence, albeit indirect, of the steroidogenic potentiality of these organisms, as it is known to generate the NADPH required for the hydroxylation of steroids during steroidogenesis.
TABLE-IX

Showing $\Delta^5$-3B-HSDH, 17B-HSDH, and G-6-PDH activities in the primites and the satellites in syzygies in trophozoite'/gamont condition.

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<td>a) Testosterone</td>
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<td>b) Estradiol 17B</td>
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The intensity of enzyme activities is graded from Minimum, +, to the Maximum; ++++; ± denotes activity in traces and - denotes no demonstrable activity.

All the chemicals are of 'Sigma grade' U.S.A.