Histochemical Studies of *Herpetomonas muscarum*

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SUMMARY

The cytoplasm of the 'leptomonas form' of *Herpetomonas muscarum* contains a parabasal body, mitochondria, and so-called 'volutin' granules. The parabasal body consists of neutral lipid (probably triglyceride), lipoprotein, and DNA. The mitochondria are in the form of granules. The volutin granules are more numerous and smaller than the mitochondria. The volutin granules appear to be composed of ribonucleoprotein. Glycogen is dispersed homogeneously in the cytoplasm. The cytoplasm is rich in RNA and protein. The nucleus is DNA-positive. Near the parabasal body lies a basal granule; a long flagellum arises from it. A flagellar vacuole lies near the base of the flagellum.

INTRODUCTION

MANY genera of Trypanosomidae (Flagellata) have been investigated previously by both light and electron microscopy.

Duboscq and Grassé (1933) homologized the parabasal body of the flagellates, consisting of chromophil and chromophobe parts, with the 'Golgi apparatus'.

Berge (1942, 1946) described small 'volutin' granules composed of ribonucleoprotein in *Trypanosome gambiense* and *T. evansi*. Anderson and others (1956) described similar volutin granules in *T. equiperdum*. Anderson and others (1956) failed to identify mitochondria in this species by electron microscopy.

Causey (1927) regarded the parabasal body of *Leishmania brasiliensis* as a derivative of the mitochondria. Sen Gupta and others (1953) described neutral red vacuoles, mitochondria, volutin granules, and kinetoplast as separate cytoplasmic inclusions in the flagellate form of *L. donovani*. The kinetoplast included a parabasal body, flagellar vacuole, and base of flagellum with a terminal blepharoplast. Chang (1956), using the electron microscope, described in *L. donovani* fine granules, vacuoles of various sizes, osmiophil lipid droplets, and certain other bodies; the latter, according to him, might be mitochondria, but their internal structure was not clear. Guha and others (1956) observed only bigger and smaller granules in this species, and identified all of them as mitochondria.

Fantham (1912) described a flagellum arising from the kinetonucleus in *Herpetomonas pediculi*. Wilson (1925) and Wenyon (1926) described in *Herpetomonas* sp. a 'cone of fibrillae' connecting the parabasal body with the blepharoplast. Grassé (1926) regarded a clear space surrounding the 'kinetonucleus' or parabasal apparatus of *Herpetomonas* as the chromophobe substance. Khajuria (1950) claimed that small granules, representing the 'presubstance' of the true Golgi material (Hirsch, 1939), were budded off from the undifferen-

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tiated lipoidal parabasal body of *H. muscarum*; they could be stained with neutral red. He denied the presence of mitochondria in this species.

Prowazek (1904), Chatton and Léger (1911), and Kofoid and McCullock (1916) described axial filaments or fibres in the cytoplasm of some species of *Herpetomonas*. Kleinschmidt and Kinder (1950) described a fibrillar structure in the periplast in *T. lewisi* and *T. brucei*; Kraneveld and others (1951) described nearly parallel, longitudinal fibrils in *T. evansi*; Meyer and Porter (1954) described sub-pellicle fibres or striation in *T. cruzi*; while Das Gupta and others (1954) observed myoneme fibrils in *L. donovani*.

Material and Methods

The 'leptomonas' forms of the flagellate *H. muscarum* (family Trypanosomidae), obtained from the gut of house-fly, were examined in physiological solution (Baker, 1944) by phase-contrast microscopy. Janus green B and neutral red were used for supervital staining.

For histochemical study, wet smears were employed. In some cases 10 μ gelatine or paraffin sections of the gut containing the specimens were employed. Details of the histochemical tests used are given in the Appendix (pp. 418–19).

Specimens were also fixed in Lewitsky, Champy, or Helly, and stained with Heidenhain's haematoxylin or with acid fuchsine (Cain, 1948*b*).

Results

The results of the cytochemical tests are set out in full in the Appendix (pp. 418–19).

The cytoplasm contains a parabasal body, mitochondria, and so-called 'volutin' granules.

Parabasal body. This appears as a duplex structure consisting of a dark externum and a light internum, in living specimens examined under the phase-contrast microscope. The externum of the parabasal body is darkened by 2% osmium tetroxide solution (fig. 1, A) and by Sudan black B (fig. 1, B); mercuric-bromophenol blue colours it (fig. 1, C). By all these techniques it appears crescentic. It appears blue in pyronin / methyl green preparations (fig. 1, D), and stains with iron haematoxylin in material fixed in Lewitsky, Champy, or Helly (fig. 1, E–G). It reacts positively to Feulgen's test.

The internum is not coloured by Sudan black even after the 'unmasking techniques' of Ciaccio (1926) or Bradbury and Clayton (1958); in fact it is negative to all the histochemical tests that were tried.

The evidence suggests that the externum of the duplex parabasal body consists of neutral lipid (probably triglyceride), lipoprotein, and DNA. Because of the presence of DNA, the parabasal body has been regarded as a secondary nucleus or kinetonucleus by Fantham (1912) and Grassé (1926) in *Herpetomonas*, and by Sen Gupta and others (1953) in *L. donovani*. The duplex nature of 'kinetonucleus' or parabasal body in *Herpetomonas* was described by Grassé (1926) also. This parabasal body, containing

DNA, cannot be homologized with the 'Golgi apparatus' as Duboscq and Grassé (1933) suggested. The composition of the internum could not be determined.

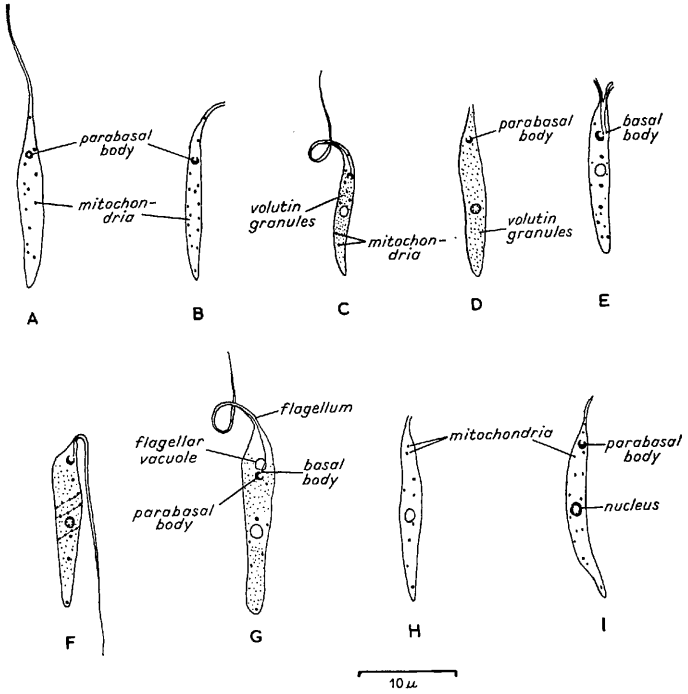

FIG. 1. Camera lucida drawings of *H. muscarum*. A, fresh specimen treated with 2% osmium tetroxide. B, formaldehyde-calcium, postchromed, Sudan black. C, weak Bouin, pyridine extraction, mercuric-bromophenol blue. D, Zenker, pyronin G / methyl green. E and F, Lewitsky, iron haematoxylin. G, Helly, iron haematoxylin. H, Helly, Cain's acid fuchsine. I, formaldehyde-calcium, mercuric bromophenol blue.

The basal body (blepharoplast) of the flagellum lies close to the parabasal body, and a flagellar vacuole lies on one side of the flagellum near its base (fig. 1, G). The basal body is stained by mercuric-bromophenol blue and also by iron haematoxylin. The 'cone of fibrillae', which connects the parabasal body with the blepharoplast according to Wilson (1925) and Wenyon (1926), could not be observed.

Mitochondria. These are in the form of granules scattered irregularly in the cytoplasm, or sometimes they are arranged along the inner surface of the

periplast (fig. 1, F). They are larger than the volutin granules. They are stainable by mitochondrial techniques (Janus green B; acid fuchsine (fig. 1, H)). They are also darkly stained in iron haematoxylin preparations (fig. 1, E–G).

Khajuria (1950) denied the presence of mitochondria in this species. Studies by electron microscopy (Anderson and others, 1956; Chang, 1956) have also shown that mitochondria possessing the typical internal structure cannot be identified with certainty in *T. equiperdum* and *L. donovani*.

Guha and others (1956) and Sen Gupta and others (1953), however, have identified mitochondria in *L. donovani* by cytochemical methods. The former authors have employed specific enzymological tests, such as oxidation of the Nadi reagent and reduction of tetrazolium; and their conclusion seems to be beyond doubt. Causey (1927) described the presence of mitochondria in *L. brasiliensis*.

It appears probable that in the family Trypanosomidae, mitochondria do not possess the typical ultra-structure that can be identified with certainty by electron microscopy. Future studies by electron microscopy will probably establish with certainty the presence of mitochondria in these forms.

'Volutin' granules. They are more numerous than the mitochondria and are evenly distributed in the cytoplasm. They are specifically stained red in pyronin / methyl green preparations (fig. 1, D). They are not coloured by mercuric-bromophenol blue in material fixed in formaldehyde or formaldehyde-calcium (fig. 1, I), but are stained darkly after extraction with lipid solvents (fig. 1, C). Fixation of protein by formaldehyde is probably incomplete (compare Baker, 1958).

The volutin granules are weakly stained in iron haematoxylin preparations (fig. 1, F–G). They are not stained supervitally with Janus green B or neutral red.

Positive reactions for RNA and protein suggest the presence of ribonucleoprotein in them. Similar volutin granules composed of ribonucleoprotein have been described in other genera of Trypanosomidae by Berge (1942, 1946), Anderson and others (1956), and Sen Gupta and others (1953).

Nucleus. The nucleus is Feulgen-positive. It shows small dots arranged along the periphery of a ring when it is stained by methyl green in pyronin / methyl green preparations (fig. 1, D).

Cytoplasm. The diffuse staining of the cytoplasm by pyronin and mercuric-bromophenol blue suggests the presence of RNA and protein. Glycogen is uniformly dispersed in the cytoplasm.

If supervital preparations are kept for a long time in neutral red, small red vacuoles begin to appear in the cytoplasm. These vacuoles appear to be new formations. Baker (1958) has described the appearance of similar vacuoles on prolonged treatment with neutral red.

Periplast. The periplast or pellicle covers the cytoplasm on all sides and shows nearly parallel longitudinal striations (fig. 1, F). Similar striations have been described by Prowazek (1904), Chatton and Leger (1911), Kofoid and McCullock (1916), Kleinschmidt and Kinder (1950), Kraneveld and others (1951), Meyer and Porter (1954), and Das Gupta and others (1954).

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APPENDIX

The histochemical reactions of Herpetomonas muscarum

Technique	*Fixation*	*Reference*	*Parabasal body*	*Mito-chondria*	*'Volutin' granules*	*Nucleus*	*Cyto-plasm*
SB in 70% ethanol	F Ca and F Ca+PC	Baker, 1956	++c	+++	–	–	–
SB in 70% ethanol at 60° C	,, ,,		++c	+++	–	–	–
SB in propylene glycol	,, ,,	Chiffelle and Putt, 1951	++c	+++	–	–	–
SB* cold acetone, ether or ethanol	Fresh or F Ca	Krishna, 1950; Pearse, 1954	+c	++	–	–	–
NB	F Ca and F Ca+PC	Cain, 1947, 1948*a*	violet	violet	–	–	–
NB* cold acetone	Fresh or F Ca	,, ,,	blue	blue	–	–	–
Sudan III and IV in 70% ethanol / acetone	F Ca and F Ca+PC	Kay and Whitehead, 1941	+c	++	–	–	–
Fettrot in 70% ethanol	,, ,,	Pearse, 1954	+c	++	–	–	–
AH	F Ca+PC	Baker, 1946	+	+++	++	+	–
AH* PE	WB and PE	,,	++	+	++	+	–
Fischler's reaction	F, F Ca	Pearse, 1954	–	–	–	–	–
Feyrter's enclosure	,,	,,	blue	blue	blue	blue	blue
Cholesterol reactions	,,	Schultz, 1924, 1925; Pearse, 1954; Gomori, 1952; Romieu, 1927	–	–	–	–	–
Performic acid / Schiff	F Ca	Pearse, 1951; Lillie, 1952	+	++	–	–	–
2% osmium tetroxide	Fresh	Nath, 1957	+r	+++	–	–	–
Ciaccio's technique	F Ca and phenol	Bradbury, 1956*b*	++c	+++	–	–	–
Flemming unmasking	Flemming's+PC	Bradbury and Clayton, 1958	++c	+++	–	–	–

PAS	B, C	Hotchkiss, 1948; Pearse, 1954	−	−	−	−	++
PAS* acetylation	,,	McManus and Cason, 1950	−	−	−	−	−
PAS* 0·1 N KOH	,,	,, ,, ,,	−	−	−	−	++
Best's carmine	,,	Pearse, 1954	−	−	−	−	++
PAS or Best's carmine* salivary amylase	,,	,,	−	−	−	−	−
MBB	F, F Ca	Mazia and others, 1953	+	++	−	+	+
MBB* cold acetone, ether or ethanol	Fresh or F Ca		++	++	++	+	+
MBB	B, C, WB+PE		++	++	++	+	+
Feulgen	C	Pearse, 1954	++	−	−	++	−
Feulgen* trichloroacetic acid	,,	Schneider, 1945	−	−	−	−	−
P/MG	Z	Jordan and Baker, 1955	blue	−	red	blue	red
P/MG* salivary ribonuc lease	,,	Bradbury, 1956*a*	blue	−	−	blue	−
P/MG* trichloroacetic acid	,,		−	−	−	−	−

KEY: AH = acid haematein; B = Bouin; C = Carnoy, c = 'crescent'; F = formaldehyde; F Ca = formaldehyde-calcium; PE = pyridine extraction; PAS = periodic acid / Schiff; PC = with post-chroming; P/MG = pyronin G / methyl green; MBB = mercuric bromophenol blue; NB = Nile blue; r = ring; SB = Sudan black B; WB and PE = weak Bouin followed by pyridine extraction; Z = Zenker; + = weak reaction; ++ = moderate reaction; +++ = strong reaction; − = negative; * = after treatment with.