Advances in
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VOLUME 32

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PREFACE

Unusually for the series as a whole, this volume of *Advances in Parasitology* has a predominantly protozoological flavour, which we hope may go some way to redressing the overall helminthological bias.

The first contribution deals with what the authors, Drs Boreham and Stenzel, refer to as an enigma: the well known but little understood *Blastocystis*. Both this organism's taxonomic position and its possibly pathogenic role remain in doubt, but Boreham and Stenzel conclude that *Blastocystis* is indeed a protist, but is probably *sui generis*—although perhaps most closely related to the amoeboflagellates. Since its first recognition by Alexieff in 1911, this organism has been variously regarded as a fungus, yeast, the cyst of a flagellate, or a degenerate vegetable cell. Opinion about its pathogenicity has been, and still is, similarly divided; the authors conclude that it is at present premature to attempt to reach a conclusion.

Drs Thompson, Reynoldson and Mendis next present a masterly review of the knowledge concerning *Giardia* which has accumulated since this parasite was last reviewed in this series in 1979 (Vol. 17). The pathogenicity of *Giardia* is not in doubt, it now being recognized as "one of the ten major parasites of humans", and nor is its taxonomic position among the flagellates. However, the increasing and intriguing (and sometimes conflicting) evidence that *Giardia* represents a very early branching of the eukaryotic stem is discussed, and the authors conclude that "some caution may be prudent" in reaching a conclusion about this. The review also deals with the relatively newly discovered intranuclear RNA virus, GLV or Giardiavirus, about which much remains to be discovered, and other aspects of the parasite's morphology, the vexed question of speciation, the life cycle and transmission (concluding that definitive proof of zoonotic transmission in nature has yet to be obtained), biochemistry (which is discussed in considerable detail), and control.

Following the review of the interaction between *Leishmania* and its macrophage host cells in the previous volume, this volume contains a full overview of current knowledge of the immunology of leishmaniasis by Professor Liew and Dr O'Donnell. Considerable advances in this subject have been made over the last decade or so, including knowledge of the genetic regulation of the response to infection in human and murine hosts, the cellular response (especially the roles of various T cell populations), cytokines and the effector mechanisms by means of which the parasites may be killed as a result of the host's response to infection. This leads to a discussion of vaccination (which has, of course, been practised for centuries

in the form of "leishmanization" for oriental sore). The authors conclude that leishmaniasis is now perhaps one of the infectious diseases best understood from an immunological viewpoint.

Dr Zilberstein reviews, in a compact and concentrated form, current knowledge of the means by which trypanosomatids transport nutrients and ions across their membranes, including proton transport and the proton motive force, and transport of glucose, amino acids and calcium. These mechanisms, which have until relatively recently been a neglected topic, are clearly of great importance in maintaining the parasites' intracellular homeostasis and thus ensuring their survival within their hosts. Equally clearly, it is possible that a fuller understanding of the mechanisms of transport could lead to the development of directed chemotherapeutic agents, aimed at blocking or disrupting these essential processes.

Dr Davies and Professor Ball next review the biology of the coccidian parasites of fish. This very comprehensive, fully and beautifully illustrated chapter complements the review of eimeriid coccidia by Professor Ball and others in Volume 28. This interesting group of organisms, which contains, in the authors' words, a "bewildering array" of parasites, has until recently been much less studied than the equivalent parasites of mammals and birds. However, the currently growing interest in fish farming and increasing awareness of the potential of these coccidia to cause disease, especially under the intensive conditions inseparable from farming, has led to a tendency to redress this imbalance—a process which will be much helped by the present review. All aspects of the parasites' biology are covered: life cycles, transmission, structure and host–parasite interactions. The authors conclude by summarizing fields in which our knowledge of these parasites has increased considerably and, perhaps more importantly, those in which it has not much increased. This latter category includes the possibility of autoinfection, immunity and pathogenicity, and taxonomy. There is still confusion and argument over the number of valid genera; alongside the well established genera *Eimeria* and *Goussia*, should the newer genera *Epieimeria*, *Epigoussia* and *Nucleogoussia* be accepted? The mechanism of oocyst wall formation also remains incompletely understood. The authors conclude optimistically that the significant recent advances in knowledge should provide the basis for controlled experiments to provide answers to these questions.

Finally Drs Raibaut and Trilles review the sexuality of parasitic crustaceans. This diverse and sometimes bizarre group of parasitic organisms is not well known to the majority of parasitologists but can supply fascinating insights into the evolutionary aspects of comparative parasitology. Parasitism has led to a multiplicity of sexual modes with separate sexes and various degrees of hermaphroditism often reflecting the motile or sessile habits of their free-living ancestors. The problems of mate encounter which occur

when one of the partners is fixed, and particularly when both are, have been solved in various ways, and these are discussed and amply illustrated.

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Blastocystis in Humans and Animals: Morphology, Biology, and Epizootiology

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I. Introduction

Blastocystis has been described as an enigma among the protists, with a number of unique features (Zierdt, 1991). This contention has certainly been true since the first accurate descriptions of this parasite (Alexeieff, 1911; Brumpt, 1912), and remains so today, largely because of the lack of critical research. *Blastocystis* has a controversial history, and progress in our knowledge has been hindered by sweeping generalizations, made from too little scientific evidence, which have been repeated without being seriously questioned. Thus, many of the dogmas currently held are based on minimal factual data, often collected more than 20 years ago, and must now be challenged. In this review an attempt will be made to differentiate between fact and speculation, with a view to putting the biology of this organism on a scientific footing and indicating the major deficiencies in our knowledge. The literature of *Blastocystis* is dominated by the work of Charles Zierdt, who must be credited with bringing this organism to the notice of medical scientists. However, his work has not been subjected to rigorous scrutiny by other scientists and does contain many inconsistencies.

The history of *Blastocystis* has recently been reviewed in detail (Zierdt, 1991), and the possible contributions of Brittan (1849), Swayne (1849), Lösch (1875) and Perroncito (1899) to the discovery of the organism appraised. However, none of the descriptions given in these early reports is entirely satisfactory and, like a number of later publications, does not exclude the possibility that the authors were looking at artefacts, such as degenerate vegetable, tissue or yeast cells. The history of the parasite will not be considered further in this review except where it impinges on current interpretation of data, and the reader is referred to Zierdt's excellent historical account for further details (Zierdt, 1991).

II. Taxonomy

Our current knowledge of the taxonomy of *Blastocystis* is grossly deficient. The history of this organism illustrates the confusion that has always existed

concerning its taxonomic position. Early workers were unable to classify *B. hominis*, and variously described it as the cyst of a flagellate, vegetable material, yeast and fungus (see Zierdt, 1978, 1991). It was not until 1967 that evidence was provided to assign *B. hominis* to the subkingdom Protozoa (see Zierdt *et al.*, 1967), based on morphological and physiological criteria. Ultrastructurally it resembles the protists as it lacks a cell wall, but contains nuclei, smooth and rough endoplasmic reticulum, Golgi complex and mitochondria. Physiologically it is anaerobic, sensitive to oxygen, fails to grow on fungal media, grows optimally at 37°C and neutral pH, and is not killed by antifungal agents such as amphotericin (Zierdt *et al.*, 1967; Tan and Zierdt, 1973; Zierdt, 1973; Tan *et al.*, 1974; Zierdt and Williams, 1974).

B. hominis was subsequently classified in the subphylum Sporozoa, in a separate suborder Blastocystina (Zierdt, 1978), and more recently in the subphylum Sarcodina (Zierdt, 1988). Molecular sequencing studies on a single human isolate (Netsky), utilizing small subunit ribosomal ribonucleic acid sequencing techniques, have shown that *B. hominis* is not monophyletic with *Saccharomyces* nor with any of the sarcodines or sporozoans, suggesting that *B. hominis* is not closely related to any of these groups (Johnson *et al.*, 1989) (Fig. 1). When the Apicomplexa were examined, only *Sarcocystis* and *Toxoplasma* were found to be monophyletic, and are separated from *Plasmodium* and *Blastocystis* by the ciliates and the dinoflagellate *Prorocentrum*. Thus, the exact taxonomic position of *B. hominis* remains undetermined, although it seems likely that eventually it will be shown to form a new group, possibly closely related and analogous to the amoebo-flagellates (see Section IV.H).

Two non-human species have recently been described: *B. galli* from the caecum of chickens in the Commonwealth of Independent States (Belova and Kostenko, 1990), and *B. lapemi* from the sea snake, *Lapemis hardwickii*, collected in Singapore (Teow *et al.*, 1991). The former was identified on morphological criteria but, due to the great variation seen between individual organisms, care should be exercised in interpreting this result until further confirmatory evidence is obtained. *B. lapemi* was differentiated from *B. hominis* on its different optimal culture requirements (*B. lapemi* growing best at 24°C rather than 37°C) and different electrophoretic karyotype.

In this review we will refer to the parasite isolated from humans as *B. hominis*, and use *Blastocystis* sp. for organisms isolated from other hosts, since there are currently no data on their interrelationship.

Intraspecific variation has been examined in *B. hominis* (Kukoschke and Müller, 1991; Boreham *et al.*, 1992). Analysis of 10 stocks of *B. hominis* isolated from human stools revealed two discrete groups of organisms. Proteins of the two groups were immunologically distinct (Figs 2, 3), and hybridization with random probes generated from the deoxyribonucleic acid

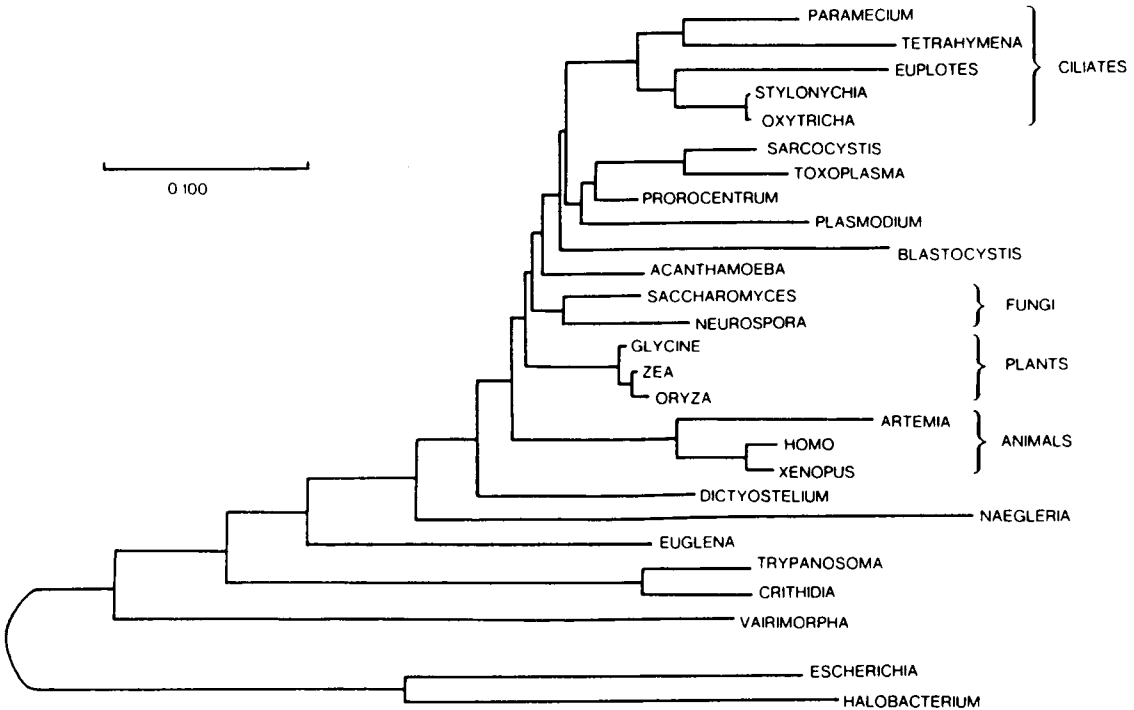

FIG. 1. Phylogenetic tree for 25 genera of eukaryotes to show the relationship of *Blastocystis* to other species. The tree is based on the degree of nucleotide divergence for 215 semi-conserved sites of the srRNA gene. Branch lengths shown are proportional to the amount of evolutionary change along each branch. (Reproduced by permission from Johnson and Baverstock, 1989.)

(DNA) of one stock showed that the DNA content of the two groups was also different (Fig. 4) (Boreham *et al.*, 1992). This raises the possibility that there are more than one species of *Blastocystis* in humans. However, without further epidemiological data it is not appropriate to designate a new species. Rather, it is germane to regard these two groups as demes in accordance with the nomenclature developed for trypanosomes (World Health Organization, 1978). Demes are defined as 'Populations that differ from others of the same species or subspecies in a specified property or set of properties'. In this case, protein and DNA criteria are used to identify demes.

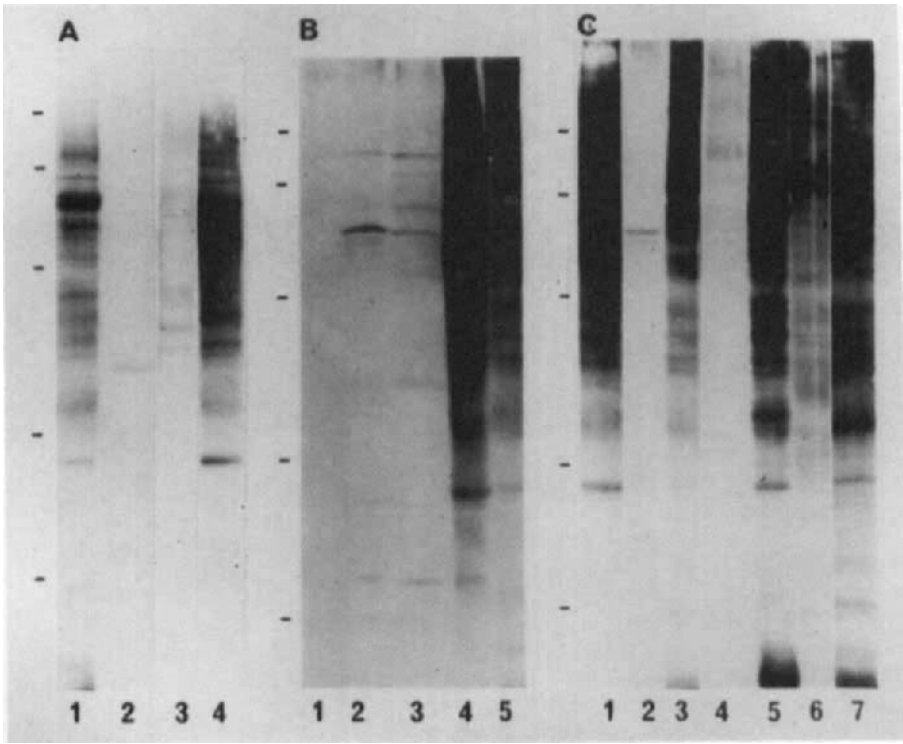

FIG. 2. Immunoblot of *B. hominis* stocks reacted with antisera raised against Netsky stock. Panel A. Lane 1, BRIS/88/HEPU/34; Lane 2, BRIS/88/HEPU/28; Lane 3, BRIS/87/HEPU/11; Lane 4, Netsky. Panel B. Lane 1, BRIS/87/HEPU/2; Lane 2, BRIS/87/HEPU/11; Lane 3, BRIS/87/HEPU/12; Lane 4, Netsky; Lane 5, BRIS/88/HEPU/23. Panel C. Lane 1, Netsky; Lane 2, BRIS/87/HEPU/11; Lane 3, BRIS/88/HEPU/23; Lane 4, BRIS/88/HEPU/28; Lane 5, BRIS/88/HEPU/32; Lane 6, BRIS/88/HEPU/33; Lane 7, BRIS/88/HEPU/34. Marker proteins are indicated in each panel and represent proteins of molecular mass 21.5, 30, 46, 69 and 92.5 kDa in ascending order. (Reproduced with permission of the Australian Society for Parasitology from Boreham *et al.*, 1992.)

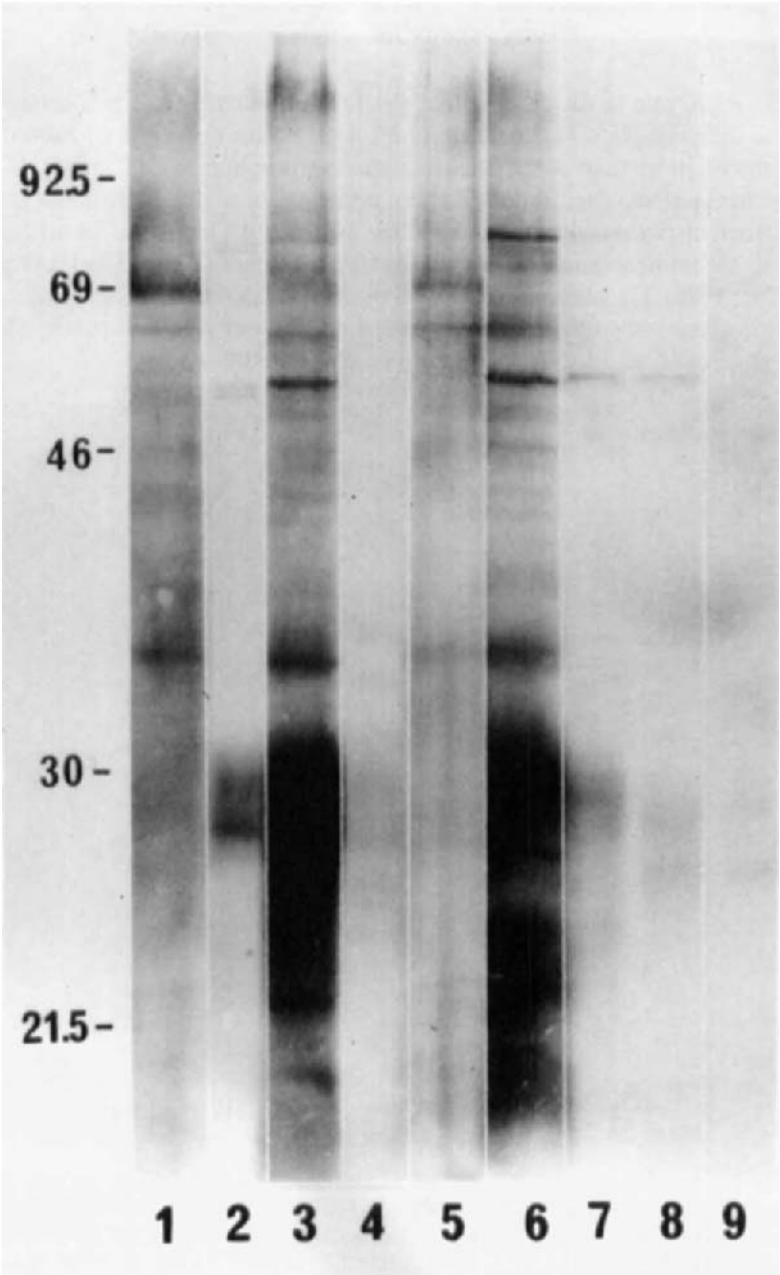

FIG. 3. Immunoblot of *B. hominis* stocks reacted with antisera raised against stock BRIS/87/HEPU/12. Lane 1, BRIS/87/HEPU/12; Lane 2, BRIS/88/HEPU/34; Lane 3, BRIS/88/HEPU/28; Lane 4, BRIS/88/HEPU/23; Lane 5, BRIS/87/HEPU/12; Lane 6, BRIS/87/HEPU/11; Lane 7, BRIS/87/HEPU/6; Lane 8, BRIS/87/HEPU/2; Lane 9, Netsky. Protein molecular masses are given in kDa. (Reproduced with permission of the Australian Society for Parasitology from Boreham *et al.*, 1992.)

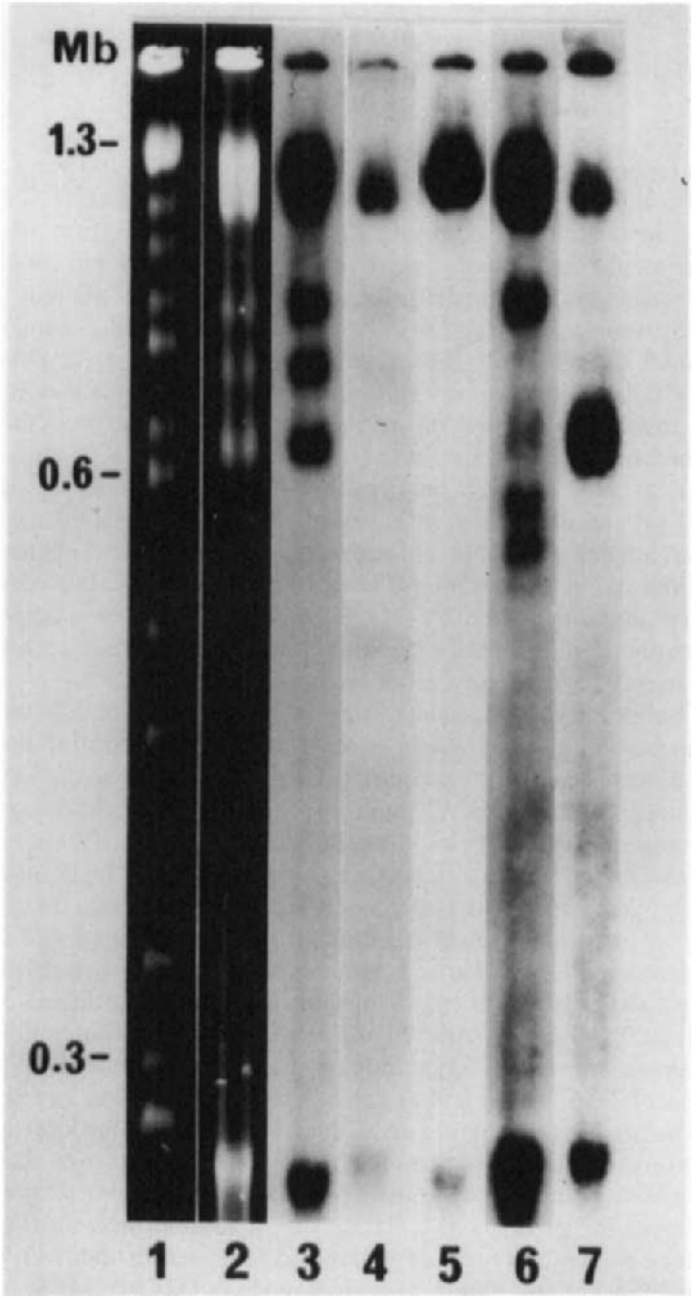

FIG. 4. Hybridization of chromosomes of *B. hominis* stock BRIS/88/HEPU/23 with DNA probes generated from the Netsky stock. Lane 1, yeast chromosome markers; Lane 2, chromosomes of stock BRIS/88/HEPU/23 separated by field inversion gel electrophoresis and stained with ethidium bromide. Lanes 3–7, chromosomes of stock BRIS/88/HEPU/23 Southern-transferred and hybridized with Netsky DNA probes. Lane 3, probe B3; Lane 4, probe B4; Lane 5, probe B37; Lane 6, probe B38; Lane 7, probe B7. Chromosome sizes are included in megabases (Mb). (Reproduced with permission of the Australian Society for Parasitology from Boreham *et al.*, 1992.)

III. Culture of *Blastocystis hominis*

The first report of the successful culture *in vitro* of *B. hominis* was in 1921, using 10% human serum in 0.5% saline with incubation at 37°C (Barret, 1921). Growth occurred mainly in the lower part of the tube where the oxygen content was reduced. Subsequently, the technique was modified to include ovarian cyst and peritoneal exudate fluids rich in albumin (Lynch, 1922). Attempts at growth on solid and semi-solid media were initially unsuccessful (Lynch, 1922; Ciferri and Redaelli, 1938), until Boeck and Drbohlav's (1925) inspissated egg medium was used (Zierdt and Williams, 1974). Growth was optimal on medium which had been pre-reduced for 48 h, at neutral or slightly alkaline pH, with incubation at 37°C. Growth did not occur at 30°C or room temperature. Axenization of stocks has been achieved with antibiotics over a period of at least 1 month, using, in particular, ampicillin, colistin and streptomycin (Zierdt and Williams, 1974). Ceftizoxime and vancomycin have been used to inhibit bacteria resistant to the former antibiotics (Zierdt, 1991). Other workers have been unsuccessful in axenization (Dunn, 1992), suggesting that in some instances the bacteria may be essential for the survival of the organism.

There have been several attempts experimentally to induce different forms of *B. hominis* in culture. Initially it was found that axenization using egg medium slants generally produced cells larger than those present in conventional cultures (Zierdt and Williams, 1974). Short-term cultivation of axenized strains on TPN broth and brain-heart infusion with 10% horse blood and an overlay of Hank's solution was found to favour the granular form (Zierdt and Williams, 1974). Other media reported to be useful for growth of *Blastocystis* sp. include Dobell and Laidlaw's medium covered with Ringer's solution containing 20% human serum and supplemented with streptomycin sulphate (Silard, 1979), Loeffler's medium covered with Ringer's solution containing 20% human serum (Silard *et al.*, 1983), and Diamond's trypticase-panmede-serum (TP-S-1) monophasic medium (Molet *et al.*, 1981). The usefulness of TP-S-1 medium has not been confirmed (Dunn and Boreham, 1991). The need for a monophasic medium for culture of the large numbers of organisms required for chemotherapeutic and molecular studies led to extensive investigation of a variety of media and conditions (Dunn, 1992). Media tested included thioglycollate broth, TYI-S-33 medium (Diamond, 1987) alone and with pyruvate, catalase and horse serum additives, medium 1640, medium 199 and minimal essential medium (MEM). MEM, containing 10% horse serum (MEMS), which had been pre-reduced for 48 h was found to be the best monophasic medium to maintain growth of *B. hominis*, although some growth occurred in medium 199.

Standardization of culture conditions enabled the measurement of dou-

bling times of *B. hominis* in culture. Zierdt and Swan (1981) found that the doubling times of eight axenic strain of *B. hominis* in biphasic egg medium ranged from 6 to 16 h, and the time required for an individual *B. hominis* cell to divide was 30–60 min. The doubling time for the Netsky strain of *B. hominis* was 19.7 ± 3.3 h in MEMS, compared to 23.4 ± 5.4 h on egg slants (Dunn and Boreham, 1991). These doubling times were considerably longer than the 8.5 h previously reported for this stock grown on an egg slant medium (Zierdt and Swan, 1981). This discrepancy may reflect selection of the parasite through continuous growth over several years, or possibly the different time period over which measurements were made.

Although *B. hominis*, unlike bacteria, cannot be stored by freeze drying (Zierdt, 1991), it may readily be cryopreserved using 7.5% dimethylsulphoxide as a cryoprotectant. Best results are obtained by controlled slow cooling and subsequent maintenance in liquid nitrogen. On retrieval from liquid nitrogen, the cells must be rapidly thawed at 37°C, washed twice in Locke's solution and inoculated into a fresh culture tube of pre-reduced medium. This allows stocks to be stored for extended periods and to be transported to other laboratories. In addition, reference material can be maintained for antigenic analysis and to ensure that continuous culture does not lead to genetic selection.

IV. MORPHOLOGY

Reports of the morphology of *B. hominis* have consistently noted several major forms (vacuolar, granular and amoeboid), and a number of less common forms, of the organism (see Zierdt, 1991). It is currently unknown whether these differences in morphology reflect differences in the biochemistry and general cell biology of the organism. The true life cycle has not been conclusively elucidated, and the differentiation pathways are uncertain. The stage of the parasite responsible for transmission is also undefined. This review on the current status of the morphology of *Blastocystis* is written with these deficiencies in mind, in order to identify those areas where research is most needed and to separate actuality from conjecture.

A. LIGHT MICROSCOPY

Many reports have described in detail the morphology of *B. hominis* as determined by light microscopy. Many of the early reports were meticulous in detail, and in general closely correlate with recent electron microscopic descriptions. Most reports described a spherical cell, with a large central