CLINICAL MICROBIOLOGY REVIEWS, Jan. 2001, p. 150–164 Vol. 14, No. 1
0893-8512/01/$04.00+0 DOI: 10.1128/CMR.14.1.150–164.2001


# Drug Targets and Mechanisms of Resistance in the Anaerobic Protozoa

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## INTRODUCTION

A small group of diverse, parasitic protozoa has significant impact on the mucosal health of humans. Historically these organisms have included *Giardia duodenalis, Trichomonas vaginalis*, and *Entamoeba histolytica*. More recently, gastrointestinal infections have been associated with *Blastocystis hominis, Cryptosporidium parvum, Isospora* spp., and *Cyclospora* spp., although these organisms are of contentious phylogeny. Collectively, they infect over one billion people each year. *G. duodenalis, E. histolytica, B. hominis*, and *C. parvum* parasitize the gastrointestinal tract, where they cause diarrhea, dysentery, and associated symptoms. *E. histolytica* can also invade the gut epithelium and subsequently other organs, where it forms abscesses which can be fatal if left untreated. *T. vaginalis* is sexually transmitted and adheres to the epithelium of the vagina, resulting in a range of symptoms from vaginitis and discharge through pelvic inflammatory disease to preterm delivery and low birth weight concomitant with increased mortality.

The basic metabolism of these protozoa appears to be anaerobic and microaerotolerant, although differing in extent in each case. Anaerobicity protects the key, low-redox-potential metabolic enzyme pyruvate:ferredoxin oxidoreductase and ferredoxin, which also activate the nitroimidazole drug metronidazole, the front line of defense against *G. duodenalis, E. histolytica*, and *T. vaginalis*. Metabolic pathways such as these, which are found in anaerobic bacteria that are also susceptible to metronidazole but not in other eukaryotes, strengthen the idea that these protozoa are very early diverging in the eukaryotic lineage. There are no recommended, successful therapeutic strategies for *C. parvum* as yet, and little is known about mechanisms of drug resistance for this parasite or for *Blastocystis, Isospora*, and *Cyclospora* spp. Less common gastrointestinal infections, particularly by other flagellates and coccidians, may also include protozoans with uncharacterized anaerobic metabolisms. This review concentrates on data which have been published or upgraded after earlier extensive reviews on specific organisms and which are quoted in the text.

## *GIARDIA DUODENALIS*

*G. duodenalis*, also known as *Giardia lamblia* and *Giardia intestinalis*, is a flagellated diplomonad and the most commonly detected flagellate and protozoan in the intestinal tract (123). The trophozoites were first noted by von Leeuwenhoek in 1681 in his own stools. A morphologically identical organism infects over 40 animal species (103) and is regarded as zoonotic by the World Health Organization (WHO). There is evidence, however, that host range can be restricted (190, 205), and there is a lack of evidence for the extent of zoonosis (209), so that animal reservoirs of human outbreaks have not been clearly identified, although they are possible (82, 91). In cold climates, the cysts which are the infective form of the parasite can survive in water sources for months (226). Although large outbreaks have been linked to contaminated water (123), the fecal-oral route is regarded as the major source of infection, particularly in countries where the water is warm or there is

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little drainage and no reticulated water supply (143). This premise is supported by the prevalence of giardiasis in child care centers and nursing homes for the elderly (39, 136, 232). The WHO estimated that 280 million people are infected each year. The incidence may be very high in children in developing countries and decreased to 2 to 7% in some industrialized nations (59, 123). Incidence varies significantly with locale (63, 65). More recently, the WHO has estimated that 3,000 million people live in unsewered environments in developing countries and the rate of giardiasis among them approaches 30%, suggesting that there are closer to 1,000 million cases of giardiasis at any one time, contributing to 2.5 million deaths annually from diarrheal disease (WHO press release, 1998).

A study of 200 patients with diarrhea in a 2-week window at a drought rehabilitation camp in Korem, Ethiopia, showed that 98% of apyrexic patients carrying trophozoites of *Giardia* or *Entamoeba* had dysentery (45). *Giardia* is the most common cause of chronic diarrhea in travelers (59, 232, 239). Although infections vary from asymptomatic through diarrhea and weight loss to profound weight loss and failure to thrive, there are currently no genetic markers or classification systems to distinguish pathogenic from nonpathogenic strains, notwithstanding various isoenzyme (6, 129, 190), DNA fingerprinting (203, 207), and electrophoretic karyotyping (220, 221) techniques to identify isolates. Nonclonal infections add to the complexity of identifying pathogenic or virulent strains (199, 207).

The organism is ingested as the inactive cyst form, which excysts to the active trophozoite form in the duodenum after passage through the acid environment of the stomach. Trophozoites graze on the duodenal or jejunal mucosa and reproduce by binary fission (1, 54, 103), after which they encyst, probably induced by passage through the local environment, including low cholesterol levels (71, 120). Detection methods in both patients and water supplies have recently been reviewed (2, 112, 123, 226).

### *Giardia* Infection

The incubation period is 1 to 2 weeks after ingestion, following which various grades of symptoms, including nausea, stomach cramps, diarrhea, and vomiting, ensue. The acute stage can last from 3 to 4 days but can persist for much longer (1). In children, a failure-to-thrive syndrome may occur (59), and in the developing world, giardiasis is an important cause of morbidity (29). Persistent infection and diarrhea may occur in immunocompromised individuals, e.g., immunoglolubin A (IgA) deficiency or variable immunodeficiency, but is less obvious in cases of AIDS (1, 59, 82).

Infestation by the organism causes decreased small intestinal brush border surface area, microvillus and villus atrophy, enterocyte immaturity, disaccharidase and luminal enzyme deficiencies, and malabsorption of electrolytes, a multifactorial pathogenesis which is not well understood (29). A number of other symptoms have been associated with giardiasis (82), but these have not been routinely or consistently found.

Similarly, granulomatous hepatitis and cholangitis have been associated with chronic diarrhea attributed to giardiasis (163). A more recent survey of 25 patients with giardiasis and high liver enzyme values demonstrated return to normal function after antiparasite therapy (182). This involvement may be consistent with poor fatty acid uptake and steatorrhea in giardiasis (59); previous anecdotal observations regarding other symptoms may therefore have a stronger foundation, but vary with the host patient (e.g., immunological status), parasite burden, or strain.

Such a benchmark example was recently described for a *Giardia* isolate which was found to be lethal for sulfur-crested cockatoos (204). This strain was genetically similar to human isolates and was infective for mice, in which it established a chronic infection with high parasite burdens; the mice also lost up to 20% of their body weight, mimicking the human failure-to-thrive syndrome (204, 205). This is the first report of a bird isolate infecting mammals. Similarly, it is not known why symptoms range from a carrier state to failure-to-thrive syndrome, although a gene was recently reported which has 57% homology with the sarafotoxins, which cause stomach cramps, nausea, diarrhea, and vomiting (37).

The *Giardia* toxin homologue is only present in some isolates and subject to the silencing of telomeric position effects, where expression can also be induced to very high levels (219). This region of the genome is also mobile among chromosomes. The gene is a member of a family of genes which vary via a cassette-like mechanism (38, 215, 220) and is not present in all isolates (219). Such complex expression patterns of genes encoding diverse toxin and cell-signaling molecules may explain the variability in symptoms.

A recent report by the European Commission (154) suggested that *Giardia* be targeted for vaccine development, which may complement the use of drug treatment.

Genes from *Giardia* were first cloned in 1987 (202). A recent review has described the plasticity in the organization and structure of the *Giardia* genome analyzed by electrophoretic karyotyping, chromosome mapping, and strain analysis, showing variable ploidy, aneuploidy, and accessory chromosomes (220). The proximal subtelomeric regions are particularly dynamic, with recombination occurring among gene blocks (telomere gene units) inserted into the repeated telomeric ribosomal DNA (rDNA) arrays and also within cassettes in cysteine-rich protein genes encoded in those gene units. The gene units are under the silencing control of a telomere position effect, are mobile within the genome, and vary among isolates (37, 219, 220).

### *Giardia* Metabolism

*Giardia* is amitochondrial and asexual. Its metabolism is glycolytic and fermentative (22, 92, 110, 131), with the terminal part of the glycolytic pathway being executed by the anaerobic bacterial homologue pyruvate:ferredoxin oxidoreductase (PFOR) replacing pyruvate dehydrogenase found in aerobic organisms (22, 196). The protein has been purified and characterized (196). ATP is generated by substrate—level phosphorylation; the tricarboxylic acid pathway found in the mitochondrion of aerobic eukaryotes is absent, although malate dehydrogenases are present. The gene for malic enzyme has been cloned (168). Electrons from PFOR are transferred to ferredoxin(s), of which three have been described (194); the major one has been sequenced and its gene has been cloned (P. Upcroft, J. A. Upcroft, D. M. Brown, and C. Kennett,

unpublished data). Some enzymes in the glycolytic pathway are dependent on $PP_i$ instead of ATP, suggesting a more primitive use of $PP_i$ bond energy (127). No cytochromes have been found in *Giardia*. A terminal oxidase which converts oxygen directly to water scavenges oxygen from this microaerotolerant organism to protect the anaerobic PFOR and ferredoxins (24). This 46-kDa NADH oxidase is the only aerobic enzyme so far described in *Giardia* and is a homologue of the enzyme found in anaerobic bacteria. This same enzyme is also found in the obligate anaerobic archaebacterium *Methanococcus jannaschii*, in which it is one of only two genes encoding aerobic functions (27), the second being NADH:ubiquinone oxidoreductase, which is also likely to occur in *Giardia*. A gene for a third likely aerobic function has recently been described for *M. jannaschii*, neelaredoxin, a small non-heme blue iron protein which is part of an oxygen sensing pathway and has superoxide dismutase activity in *Desulfovibrio gigas* and other anaerobes (178). In *Giardia*, PFOR is assisted by another 2-oxoacid oxidoreductase and by an arginine dihydrolase pathway to provide alternative sources of energy (22, 98, 196, 216); the gene encoding one of these eubacterial-like PFORs has been cloned (196). The gene for acetyl coenzyme A (acetyl-CoA) synthetase has been cloned and is a member of a family of similar genes of limited taxonomic distribution (169). A malate dehydrogenase gene representative of the eukaryotic cytosolic group has been cloned and is related to the same gene from *T. vaginalis*, leading to the suggestion that mitochondrial properties were lost in the common ancestor of both groups (165); an alternative and more parsimonious interpretation is that similar cell fusions generated the first eukaryotes and *Giardia* retained predominantly the anaerobic gene repertoire, while others pursued the aerobic, mitochondrial evolutionary route (220). Final endproducts of fermentation in *Giardia* are $CO_2$, acetate, alanine, and ethanol, which vary according to growth conditions (22, 50, 145).

The conventional mechanisms of oxidative stress management, including superoxide dismutase (SOD), catalase, peroxidase, and glutathione cycling, are absent from *Giardia*. They are replaced by a broad-range prokaryotic thioredoxin reductase-like disulfide reductase and the low-molecular-weight thiols cysteine, thioglycolate, sulfite, and CoA, the NADH oxidase, and a membrane-associated NADH peroxidase (22). Genome sequencing projects will probably yield information for other genes encoding functions which have not been assayed yet or have no assays, like the neelaredoxin of *M. jannaschii*. This information will complement the biochemical data, since the 30 to 50% novel genes found in each of the genomes so far studied suggest that functional studies will remain necessary to understand the diversity of biochemistry in most organisms. To draw an extreme analogy in an era of frenzied evolutionary hypothesizing, it is of note that *M. jannaschii* also appears to have a genome of variable ploidy (122).

### *Giardia* Drug Treatment and Resistance

*Current Medical Diagnosis & Treatment 1999* (191) lists tinidazole, metronidazole, furazolidone, and quinacrine for treatment of giardiasis in the United States, with success rates of less than 90% (furazolidone less than 80%), followed by albendazole (along with paromomycin), with success rates of 10 to 95%, as a possible treatment. Similar recommendations are found in *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases* (82), the *Oxford Textbook of Medicine* (81), and *Harrison's Principles of Internal Medicine* (136) (Fig. 1).

Quinacrine was the first effective antigiardial (79) until it was supplanted by the nitroimidazole metronidazole (218). Quinacrine is a substituted acridine and was introduced as an antimalarial in the mid-1930s. Quinacrine is not recommended in Australia because of its side effects, although it is still used in France and has been used effectively in cases of nitroimidazole failure (200). Metronidazole has been regarded as the drug of choice, with the derivative tinidazole being an alternative (18). Single-high-dose regimens and regimens lasting 3, 5, and 7 days with lower doses have variously been recommended (18, 191). Clinical resistance prevalence levels as high as 20% have been reported (19, 59), with recurrence rates as high as 90% (240). Resistant organisms have been isolated from patients refractory to metronidazole and the nitrofuran furazolidone; there is a large range of susceptibility to these drugs, and the development of resistance has been demonstrated in patients (59, 208, 218; L. Favennec, personal communication). Cross-resistance to tinidazole has been demonstrated with metronidazole-resistant *Giardia* strains (208, 218) (Fig. 2).

Furazolidone was often recommended for children because of its availability as a suspension (18, 93). Furazolidone-resistant *Giardia* strains developed in vitro adapted more readily to quinacrine resistance (200). Furazolidone is not available in some countries, including Australia. Metronidazole, quinacrine, and furazolidone are not tolerated with alcohol because of a disulfiram-like reaction (93). Tinidazole is not available in the United States.

Albendazole was first introduced in 1982 as an anthelmintic. The first published report of (successful) use of albendazole against *Giardia* was in 1986 (243, 244), with other benzimadazoles also being tested prior to 1990 and having mixed success (18). The first large-scale human study was in Bangladesh, where the average efficacy of albendazole was 62 to 95%, compared with 97% for metronidazole (77). The higher levels of efficacy with albendazole require multiple doses. These figures are consistent with recent, smaller studies and reports of albendazole treatment failures (21, 99). The single high doses of metronidazole or tinidazole are an advantage for compliance. Albendazole eventually may be accepted as an alternative to metronidazole, particularly in cases of metronidazole failure, if current levels of albendazole resistance or treatment failure do not increase further. Like metronidazole, resistance of *Giardia* to albendazole has been reported to occur readily in vitro (111, 206). Albendazole resistance also developed more readily in a furazolidone-resistant strain, and therefore these strains are multidrug resistant (206, 218). A giardiasis patient with parasites refractory to metronidazole and albendazole administered separately was successfully treated with these drugs administered together (unpublished data). Toxicity and side effects have been discussed at length (e.g., references (18 and 93).

Other drugs have been used to treat giardiasis with lower levels of success (18, 166). New 5-nitroimidazoles have been developed in anticipation of further deterioration in susceptibility of clinical cases to the few effective drugs available (201).

Purified *Giardia* PFOR and ferredoxin have been shown to activate metronidazole in vitro (193, 194, 196, 218). PFOR is

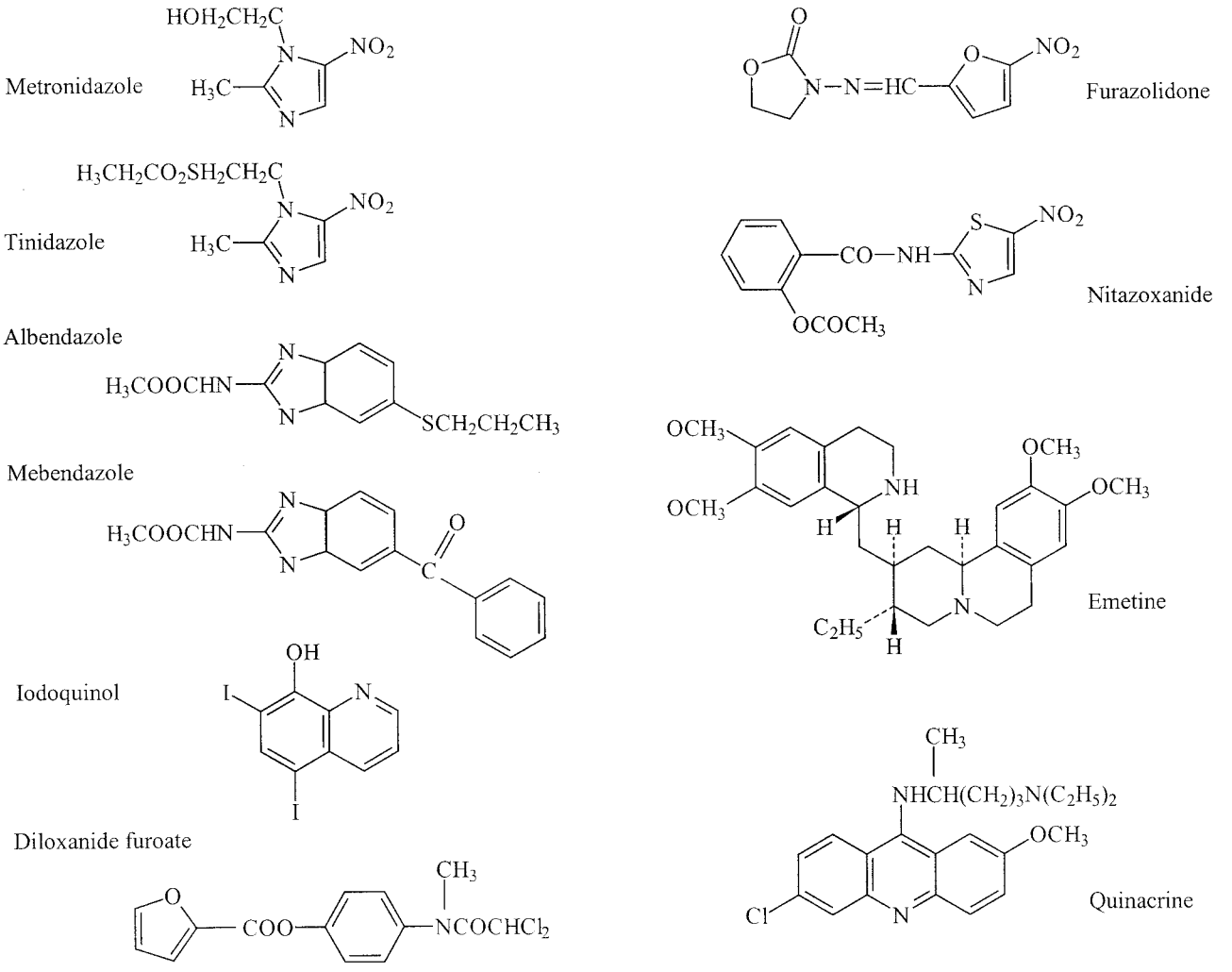


FIG. 1. Structures of the drugs commonly used against the anaerobic protozoa. Structures are redrawn from the Merck Index except for nitazoxanide, which was redrawn for direct comparison with the nitroimidazoles, nitrofurans, and benzimidazoles (166).

downregulated up to fivefold in metronidazole-resistant *Giardia*. This is consistent with the reduction in activation of the drug to its nitroso and other radicals in anaerobes by low-redox-potential electron transfer reactions (196). PFOR was not downregulated in furazolidone-resistant *Giardia*, and this strain was not affected by quinacrine. The next electron acceptor in the transport chain, ferredoxin I, is also downregulated about sevenfold, determined by in vitro assay in the same strain, but only twofold by Western blotting, suggesting that another component may be regulating the acceptor activity in the cytosolic extract (117, 218). In *Helicobacter pylori*, PFOR appears to be downregulated by mutations in an NADPH nitroreductase which also render the bacterium metronidazole resistant (73). Increased efflux of metronidazole is also involved in protecting the parasite (208, 215–218). PFOR is not totally downregulated, as in highly resistant *T. vaginalis*, probably because the enzyme is essential to the glycolytic pathway. In vivo assays suggested that PFOR is downregulated further than the assays executed on extracted enzyme (51, 52), implying that a second level of enzyme control may also be acting in the resistant cells (196, 218).

A second 2-oxoacid oxidoreductase supplants the reduced PFOR activity (196, 216). This activity has different substrate requirements, principally 2-ketobutyrate, does not neccessarily donate electrons to ferredoxins, and appears to bypass the activation of metronidazole because of a higher redox potential (216). Albendazole resistance was easier to induce in metronidazole-resistant *Giardia* (206), producing a multidrug resistance phenotype. Although the cytoskeletal structure was changed in albendazole-resistant lines, the common mutation seen in other organisms resistant to albendazole in β-tubulin at amino acid position 200 was not observed, suggesting an alternative mechanism of resistance.

Furazolidone resistance has also been induced in *Giardia* (22, 195), and NADH oxidase has been shown to activate it to its free radical state (22, 24). These lines were also more easily induced to quinacrine resistance, generating a second type of multidrug resistance (200). Although it has been suggested that the primary mode of action of quinacrine is DNA binding, the innate fluorescence of quinacrine was used to show that it does not target the nucleus of *Giardia* (200). Sensitive cells showed cytoplasmic membrane fragility. It is actively excluded from

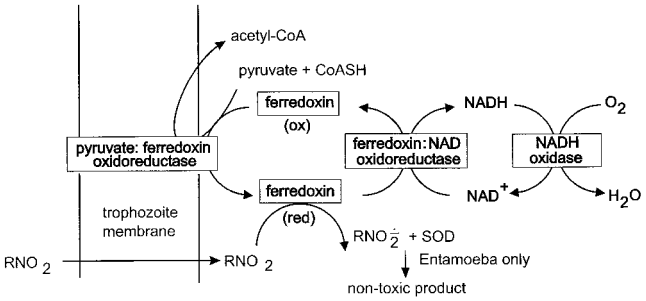


FIG. 2. Proposed fundamental metabolism of the anaerobic protozoa, with PFOR replacing the pyruvate dehydrogenase of aerobic organisms. Electrons derived from oxidative decarboxylation of pyruvate (catalyzed by PFOR) are transferred to ferredoxin (oxidized [ox]), which subsequently donates electrons to $NAD(P)^+$ (catalyzed by putative ferredoxin:NAD oxidoreductase), regenerating intracellular pools of NAD(P)H and oxidized ferredoxin. In the process of ferredoxin reduction, acetyl-CoA is generated from pyruvate and reduced CoA (CoASH). Alternatively, ferredoxin has a sufficiently low redox potential to reduce (activate) metronidazole ($R\text{-}NO_2$) to its toxic nitroradical $RNO_2^{\cdot-}$ via reduced (red) ferredoxin (194, 196). NADH oxidase then serves as the terminal oxidase in the transfer of electrons to $O_2$, producing $H_2O$. NADH oxidase also has the potential to reduce furazolidone to its toxic nitro radical. Adapted from *Giardia* data in references 22 to 25. The PFOR reaction occurs in the hydrogenosome in *T. vaginalis*. There are likely to be other coupled reactions, including alternative oxidoreductases, ferredoxins, and flavodoxins (22, 25, 117, 194, 216).

quinacrine-resistant *Giardia* cells and inhibits NADH oxidase (23), the same enzyme that activates furazolidone.

## *ENTAMOEBA HISTOLYTICA*

The trophozoite stage of *E. histolytica* was first described by Lösch in 1875, and the cyst form was first described by Quincke and Roos in 1893 (109, 123, 157). The cysts survive in water and food and are the infective form upon ingestion. At neutral or slightly alkaline pH, the cysts turn into active metacystic trophozoites, which mature into trophozoites in the large intestine (123). The cycle is completed by formation of cysts in the intestinal tract.

Approximately 500 million people are infected with *E. histolytica* at any one time (173). About 10% of these have clinical symptoms, while in approximately 10% of these cases the parasite invades the intestinal mucosa. The WHO estimates that *Entamoeba* causes severe disease in 48 million people each year, killing 70,000 each year (229), while other estimates place this figure closer to 100,000 (157, 173). Recently, the differentiation of *E. histolytica* into two species, the nonpathogenic and noninvasive *E. dispar* and the pathogenic and invasive *E. histolytica*, originally proposed by Brumpt in 1925, has regained acceptance, based principally on isoenzyme typing (48, 157) but supported by gene sequencing data (143). This conclusion is also supported by ultrastructural and cytopathogenicity studies on two representative strains (55). One interpretation of this new classification is that every individual infected with *E. histolytica* would suffer disease symptoms of dysentery involving invasion of the intestinal mucosa, while in up to 20% the parasite invades beyond the mucosa to other internal organs, including the liver and brain, assuming that half the infections are caused by *E. histolytica*, although this may be closer to one third in some areas (78). If untreated, brain abscesses, and up to 20% of cases involving abscesses in other organs, are fatal. This classification appears to be a very reasonable working interpretation based upon the disease symptoms. If facets of pathogenicity are later found to be graded between the two species or other intermediate "species" are found, appropriate reinterpretation or subclassification may need to be considered, e.g., a high rate of occult infection with *E. histolytica* has recently been observed in Mexican children without dysentery, using analytical tools which reinstated the *E. histolytica* and *E. dispar* species (139) Earlier work also suggested a large range in the frequency of asymptomatic infections with *E. histolytica*, the mechanism of which remains unknown (157). Similarly, an asymptomatic carrier in Italy appears to have infected five family members, one of whom died (67).

### *Entamoeba* Infection

The incubation period after ingestion of cysts can vary from days to months or even longer. Symptoms can range from acute amoebic colitis, which usually presents as frequent bloody stools over several weeks with fever, through profuse bloody diarrhea with fever, to amoeboma with dysentery, and finally invasion to other organs. This last stage has been equated with metastasis of colon tumors (106). Progress towards a possible vaccine has recently been reviewed (183).

Genes for cytoskeletal proteins, dominant antigens, multidrug resistance glycoproteins, adhesion molecules, including the Gal/GalNac lectin (157), and lytic factors have been cloned (125). An extracellular matrix binding protein gene encodes a multifunctional alcohol dehydrogenase and acetaldehyde dehydrogenase, homologous to the *adhE* gene of *Escherichia coli* (26). Attempts at defining an electrophoretic karyotype have had mixed success (10, 11, 47, 148), with the most recent data suggesting variation among isolates, polyploidy, and 31 to 35 chromosomes ranging from 0.3 to 2.2 Mb (231). rDNA appears to be on extrachromosomal circles (47), but it has been proposed that rDNA is chromosomally located as well (11).

### *Entamoeba* Metabolism

*Entamoeba's* metabolism appears similar to that of *Giardia* and *Trichomonas*, that is, anaerobic, fermentative, and bacterial in nature, but lacking the hydrogenosome organelle of the latter organism (158). However, *Entamoeba* has been described as a facultative aerobe in that it can grow in 5% oxygen (125). This may be better described as aerotolerant or microaerotolerant, because we were unable to find any publication that demonstrated growth rate improvement aerobically. Oxygen and its breakdown products are toxic to *Entamoeba* (13, 70), and at 5% atmospheric oxygen there is very little oxygen in the medium itself because it is reduced (13). The parasite produces SOD, catalase, and peroxidase for detoxification (36, 187). Many of the glycolytic enzymes are reversible (127, 158) and utilize the energy of the pyrophosphate bond, not ATP. To date, only one 2-oxoacid oxidoreductase, PFOR, has been detected in *Entamoeba*, and it is predominantly membrane bound (167, 189, 216). This enzyme also utilizes alternative substrates such as α-ketobutyrate, α-ketoglutarate, and oxaloacetate. The gene encoding PFOR has been cloned (164,

189). Unlike *Giardia, Entamoeba* produces SOD, and two different SOD activities have been detected (167). *Entamoeba* lacks glutathione reductase activity and does not synthesize glutathione (57). The major low-molecular-weight thiol is cysteine, although it has recently been shown that *Entamoeba* can take up glutathione from the medium and convert it to bisglutathionyl-spermidine (trypanothione) (142), a thiol derivative first found in trypanosomes (58). Novel cysteine synthase genes have been cloned from *Entamoeba* (140). An *Entamoeba* organelle of unknown function to which the chaperonin Hsp60 homes has recently been described (121, 192); whether this represents a degenerate mitochondrion, a novel organelle derived from an alternative symbiosis, or the product of an early fusion(s) to generate eukaryotes remains to be determined. An example that multiple symbioses and/or fusions can be considered is the weevil *Sitophilus oryzae*, which has three intracellular organelle genomes, mitochondrial, the γ3-proteobacterial *S. oryzae* principal endosymbiont, and the α-proteobacterial *Wolbachia* genome (80). Such symbioses appear to be quite common in biology, even among bacteria (41).

### *Entamoeba* Drug Treatment and Resistance

Emetine was first demonstrated to be an effective amoebicide in 1912 (97). A miscellany of drugs in addition to emetine have since been reported to be effective to various degrees. Metronidazole has become the drug of choice following recognition of its amoebicidal properties in the mid-1960s.

Emetine (or dehydroemetine) hydrochloride is a potent tissue amoebicide. Although it continues to be a life-saving drug in appropriate situations, this drug does have serious side effects (97). The drug is slowly excreted into the gut and urine, allowing high concentrations to build up in the liver, heart, and other viscera. To illustrate, emetine may be detectable in urine 40 days after the termination of treatment. Emetine is administered by daily intramuscular or deep subcutaneous injections for a maximum of 10 days, with local pain and tenderness at the sites of injection being common. If given orally, the drug usually causes vomiting, and very little is absorbed. Given intravenously, it is dangerously cardiotoxic (97).

There is evidence that multidrug resistance transporters may be involved in emetine and colchicine accumulation in a drug-resistant selected line, because uptake was lower in the resistant line and verapamil increased drug accumulation to that seen in the parental line (9, 20). Six P-glycoprotein-like genes have been cloned: four are expressed in a drug-resistant line, and there is differential expression (72).

The synthetic 5-nitroimidazole metronidazole, on the other hand, is remarkably safe in comparison to emetine and is now the recommended drug for the treatment of amoebiasis. It is also effective against a wide range of anaerobic bacteria (64) because of similar low-redox-activating enzymes. Oral doses of metronidazole are readily absorbed and can be found in most body fluids with few side effects. It is now so widely used both therapeutically and prophylactically for numerous major and minor ailments that exposure of *Entamoeba* to this drug worldwide is guaranteed. Inappropriate short-term exposure and exposure to sublethal levels of metronidazole are normally prescribed for prophylaxis and are precisely the conditions under which drug resistance is induced (208).

Indeed, using stepwise incremental increases in drug dose, we have induced metronidazole resistance in two axenic lines of *E. histolytica* (167). Parasites of strains HM-I:IMSS (isolated from a rectal ulcer from an adult male in Mexico City in 1967) and HTH-56:MUTM (established in culture from an amoebic abscess from an adult in Bangkok in 1992) currently grow in our laboratory in 10 μM metronidazole, a concentration of drug which is normally lethal to parasites in vitro (68). A minimum lethal dose for this strain has been reported as 11.6 μM over 72 h, and a 50% inhibitory dose of 29.7 μM has been reported, while the reported susceptibility of the HK-9 strain ranges from a 50% effective dose of 2 μM to a lethal dose of 11.6 μM over 100 h (167). Expression of the iron-containing SOD was increased three- to five-fold in metronidazole-resistant parasites, and a second SOD activity disappeared (167). Unlike in *Giardia* and *Trichomonas*, PFOR activity did not decrease significantly. The work with the iron-containing SOD has been confirmed at the gene expression level by Wassmann et al. (227), with parasites resistant to 40 μM metronidazole; peroxiredoxin expression was also increased, and ferredoxin 1 expression was decreased.

Unique in that it is effective both in the bowel lumen and in tissues, metronidazole has been reported to eradicate only up to 50% of luminal infections (191). This statement has support from a study of 36 patients with amoebic liver abscess for whom the hepatic lesions were cleared; but 20 were recolonized in the intestine, 16 asymptomatically. This was ascribed to the pharmacokinetics of metronidazole cycling in the liver and the action of metronidazole against trophozoites but not invariable eradication of cysts, creating *E. histolytica* carrier states (90, 93). These figures are considerably higher than generally published, although no single treatment has claims of 100% efficacy; 90% success after 5 to 10 days of metronidazole treatment is regarded as adequate by some workers. Alternatively, other workers have regarded that this level of efficacy suggests resistance and the need for multiple drug treatment (157). Because the organism is difficult to culture axenically from patients, there are insufficient reliable data regarding resistance levels, although a recent study indicates decreased susceptibility in clinical isolates from Chandigarh (R. Sehgal, unpublished data). Therefore, current recommendations suggest the use of metronidazole or tinidazole plus the luminal amoebicide diloxanide furoate or iodoquinol, with other combinations (including paromomycin, tetracycline, and chloroquine) depending on the severity of the infection and site, i.e., whether it is intraluminal, invasive, or abscessed (157, 191). Iodoquinol and diloxanide furoate have unknown mechanisms of action (93). Like diloxanide furoate, paromomycin is poorly absorbed. There are no reports of the very high levels of resistance to metronidazole found in *Trichomonas* or *Giardia*.

Similar to the interpretation of pathogenic versus nonpathogenic strains or species of *Entamoeba*, coupled with treatment failure due to resistance, there is disagreement over which cases should receive treatment. Clearly, anyone with serious disease requires adequate treatment with the available drugs. It has been strongly argued that metronidazole should not be used prophylactically to preserve the usefulness and potency of the drug (76, 208, 218). Sargeaunt (170) is quite adamant that only *E. histolytica* is pathogenic, obviating the need to treat *E. dispar* carriers, while Diamond and Clark (48) caution

against witholding treatment from asymptomatic individuals because *E. histolytica* has been found in these carriers. Burchard (28) advises treatment of *E. histolytica* only, but that all cyst passers be treated in the absence of differentiation. Results of a treatment and placebo trial in Mexico were interpreted to mean that money spent treating carriers in this country could be better spent on treating cases of amoebic disease (66). All these opinions appear to be correct in their context, in the absence of adequate funds and new, effective antiprotozoal drugs, and in lieu of eradication.

## *TRICHOMONAS VAGINALIS*

Trichomoniasis is a common, sexually transmitted disease caused by the flagellated protozoan parasite *T. vaginalis* (160). *T. vaginalis* was first described by Donné in 1836 (147). The trophozoites have four free anterior flagella (one along the outer edge of an undulating membrane) and one recurrent; there is no known cyst form. Trophozoites divide by binary fission (30). The most frequent form of nonvenereal infection is perinatal.

The WHO has estimated that 180 million infections are acquired annually worldwide. The estimates for North America alone are between 5 and 8 million new infections each year, with an estimated rate of asymptomatic cases as high as 50% (87, 147). *T. vaginalis* has been isolated from 14 to 60% of male partners of infected women and 67 to 100% of partners of infected men. Up to 58% of young men at risk for sexually transmitted diseases are reported to have been infected (172). In Nigeria, 37% of female students at a higher education institute were recently reported to have trichomoniasis (7), which is comparable to the data from an American study (172). Institutional crowding in itself is insufficient for transmission (96). In rural South Africa, 65% of pregnant women attending an antenatal class were reported to have trichomoniasis in 1981 (88, 171) and 49% in 1989 (141). In another more recent South African rural survey, this figure was 41% (230). Among Australian aboriginals from whom genital swab samples had been taken, 17% were positive for trichomonads, while the rate was less than 1% for nonaboriginals (225). The prevalence of trichomoniasis in women attending a sexually transmitted diseases clinic in Ulaanbaatar, Mongolia, was 67% in 1998 (175). Thirty-three percent of men at a sexually transmitted disease and dermatology clinic in Malawi were *T. vaginalis* positive, of whom 20.8% were symptomatic and had a sixfold increase in human immunodeficiency virus (HIV) concentration in their semen (83, 87). These data emphasize earlier suggestions of the association between HIV/AIDS and trichomoniasis (4, 56, 128, 181).

Trichomonal infection in women ranges from an asymptomatic carrier state to profound, acute, inflammatory disease (160). The parasite principally infects the squamous epithelium of the genital tract but can be recovered from the urethra and has been found in the fallopian tubes and the pelvis (74, 147). In males, *T. vaginalis* causes urethritis and prostatitis (100). Pneumonia, bronchitis, and oral lesions have also been well documented. Respiratory infections are acquired perinatally from infected mothers (86). In children, trichomonads can infect the urinary tract as well as the vagina. *T. vaginalis* infections have been linked to sterility problems, but there are no unequivocal data, and to adverse pregnancy outcomes (4). On the other hand, *Trichomonas foetus* in cattle causes infertility, resulting in considerable economic loss (40, 84). Recent, prospective epidemiological studies have shown that among 14,000 women examined in university-affiliated hospitals in the United States, *T. vaginalis* infection is associated with low birth weight and preterm delivery (40%) (42, 171). These in turn are closely associated with high mortality, particularly in the black minorities. *T. vaginalis* infection also predisposes carriers to HIV/AIDS (4, 128, 181), and a study of 19,000 women in Finland has recently indicated a high relative risk of cervical cancer with *Trichomonas* infection (223).

*T. vaginalis* uses lectin-like adhesins to attach to the epithelial mucin, proteases to degrade the mucin, and flagellar motility to colonize the underlying epithelial cells (53, 104). A number of adhesin genes have been cloned and appear to encode adapted metabolic enzymes. Early studies indicated considerable morphological variety among *T. vaginalis* isolates (85). This variety was supported in limited studies of protease diversity (137), a 270-kDa surface protein that varies phenotypically (3), and monoclonal antibodies to surface antigens (61), but does not appear to have been pursued in any further detail.

The karyotype of *T. vaginalis* has been described as haploid, with six chromosomes (49), and diploid, with 12 chromosomes (238). Although high-molecular-weight DNA has been extracted and genes have been cloned, no consistent electrophoretic karyotype has been described because of apparent DNA degradation during lysis block preparation, unusual chromosome topology, or large size preventing migration into the agarose.

### *Trichomonas* Metabolism

*T. vaginalis* lacks mitochondria and other characteristics of higher eukaryotic respiratory metabolism such as cytochromes and oxidative phosphorylation (51, 52). Nutrients are taken up by transport through the cell membrane and by phagocytosis. Energy requirements are provided by glycolysis of glucose to glycerol and succinate in the cytoplasm, followed by further conversion of pyruvate and malate to hydrogen and acetate in an organelle called the hydrogenosome (132, 133). Lactate dehydrogenase is present; the gene has been cloned and is derived from the gene for malate dehydrogenase (234). The hydrogenosome contains electron transport components linked to PFOR, hydrogenase, and an undefined oxidase (terminal?). Hydrogenosomes also store calcium (89). The hydrogenosome may be a degenerate mitochondrion (15, 132, 133), an organelle derived from a symbiosis with a bacterium related to those that generated mitochondria (95, 124, 130), or a relic of early fusions between groups such as the archaebacteria and eubacteria (75) that generated the earliest eukaryotes (220). Sequencing of the genome of the α-proteobacterial, intracellular parasite *Rickettsia prowazekii*, phylogenetically closely related to the originator of the mitochondrion, casts doubt on the first two possibilities, since its reduced genome does not contain the anaerobic pathways found in hydrogenosomes (134). This leaves the origin of the hydrogenosome different from that of the mitochondrion, via another type of genome reduction not found in *Rickettsia* today, or as a product of multiple, transitory, or ephemeral symbioses being tested in the early

fusions that generated the first eukaryotes (220). *Mycoplasma hominis* has been reported to be a common parasite of *T. vaginalis* also (152). Although this interpretation has been challenged (188), there appears to be a strong association of these two organisms (153), which suggests that symbioses may have been common in the evolution of *Trichomonas*.

Although *Trichomonas* is regarded as an anaerobe, there is a report that growth rates increase at levels of oxygen that are barely detectable by mass spectrometry (146). These levels of oxygen (0.025% atmospheric oxygen) are in the range that *E. coli*, for example, has switched from aerobic, through scavenging, terminal cytochrome oxidases, finally to anaerobic metabolism (197). It is unclear why an organism with such exquisite sensitivity to increased growth rates at such low oxygen concentrations has not exploited the concomitant potentially large increase in ATP production demonstrated in the other aerobes. On the other hand, growth response appears to be far more sensitive to $CO_2$ concentration (146), which suggests that metabolic pathways are present that have not been defined as yet; extremely low $O_2$ utilization may reflect coupled scavenging or protection pathways that are not revealed in the culture conditions currently employed. A more recent paper suggests that oxygen is converted into hydrogen peroxide via a cytosolic NADPH oxidase (35). There is no evidence for $O_2$ utilization in *Giardia*, although $O_2$ has been shown to be consumed (110, 229). Alternatively, it has been shown that $O_2$ can be converted directly to $H_2O$ by NADH oxidase to protect the key anaerobic enzymes in the glycolytic pathway, PFOR and ferredoxin, for example (22, 24). These components have been purified and their activity has been demonstrated in vitro (194, 196). It is of interest that the strict archaebacterial anaerobe *M. jannaschii* has only two aerobic enzyme genes encoded in its genome (27), one of which is NADH oxidase, which is closely related to the giardial enzyme (196). Purification of the appropriate metabolic enzymes from *Trichomonas* may resolve our understanding of the possible roles of $O_2$ in its metabolism and in metronidazole resistance (see below).

## *Trichomonas* Drug Treatment and Resistance

Although *T. vaginalis* was discovered in 1836 and has been known to cause vaginitis since 1916, it was not until 1957 that an effective cure, metronidazole, was discovered. Drug resistance was first reported soon after in 1962 (118), although metronidazole is still the drug of choice (94, 177). Cross-resistance between different nitroimidazoles has been reported (135) and is consistent with earlier studies (126, 135). Metronidazole-resistant isolates were sensitive to the nitrofuran furazolidone (135), consistent with these drugs not being cross-resistant in multidrug-resistant *Giardia*. A metronidazole-resistant clinical isolate has been described which shows only partial cross-resistance to tinidazole (224).

There has been reluctance to utilize metronidazole for trichomoniasis in pregnant women, particularly in the United States, because of weakly mutagenic effects in bacteria and carcinogenic effects detected in rodents (43, 44, 171, 180). However, no teratogenic effects have been detected during the long use of metronidazole in humans, and there is an easing of this stand because of the association of trichomoniasis with preterm deliveries, low birth weight, and increased infant mortality (42, 171) and predisposition to HIV/AIDS (4, 128, 181). It is now regarded as acceptable to use metronidazole, particularly in the last two trimesters of birth, to treat trichomoniasis, and possibly in the first trimester if warranted (31, 42, 43, 44, 149, 171, 174, 177).

### Aerobic versus Anaerobic Drug Resistance

Although there is circumstantial evidence for changes in the structure of the hydrogenosome in drug-resistant *T. vaginalis*, it is unclear whether they are primary events or secondary to the advent of resistance (94). Earlier work suggested that the hydrogenosome could be lost in drug-resistant cells, but recent data have shown that the organelle remains in a modified form (J. Kulda, unpublished data). There is, however, evidence for changes in upstream regulatory regions of the ferredoxin gene in four drug-resistant strains (151). Intracellular levels of the ferredoxin protein and its mRNA were reduced by approximately 50%; whether this is adequate to confer resistance is not known. A Czechoslovakian study in 1988 showed a decrease in PFOR and hydrogenase activity, but it is unknown whether these are primary or pleiotropic events (33, 34). Very few new data have appeared since (52). In principle, both PFOR and hydrogenase could activate the 5-nitroimidazoles via a ferredoxin or perhaps flavodoxin intermediate.

It has been proposed that clinical cases of drug-resistant *T. vaginalis* occur as aerobic resistance because $O_2$ is required in some way to detoxify metronidazole (33, 34, 52); resistance has been accounted for as a decreased affinity for $O_2$ by the respiratory system. Results of electron spin resonance spectrometry (235) suggested that one metronidazole-resistant isolate was deficient in $O_2$ scavenging. Further work with membrane inlet mass spectrometry indicated a diminished affinity for $O_2$ by a hydrogenosome-containing fraction. However, the most recent data (52) indicate that hydrogenase activity is lowered in two clinically resistant isolates, which is a phenomenon (along with decreased PFOR activity) previously attributed to anaerobic resistance (33, 34, 94). Furthermore, the proposed decrease in oxidase activity is inconsistent in these isolates and the decrease in $H_2$ production is also inconsistent with earlier work (33, 34). Yarlett et al. (235) suggested that decreased oxidase activity associated with the hydrogenosome could be responsible for a possibly higher $O_2$ concentration inside the hydrogenosome; the $O_2$ could then cycle with the reduced free-radical forms of the nitroimidazoles, decreasing their effective concentration. In the most recent work by the same group (52), in which the changes in oxidase levels are modest compared with the hydrogenases, one of the methods for the purification of the NADH and NADPH oxidases also included the much more abundant cytoplasmic oxidases (113) that apparently are not correlated with resistance (33, 34). It is therefore unclear at this stage whether a discrete hydrogenosome-specific oxidase activity is involved with drug resistance. The mechanisms proposed for drug resistance in general need clarification with standardized techniques and common strains.

As resistance levels increase in parasites that have been selected for resistance by increasing the levels of metronidazole that they will tolerate, there is a progressive decrease in PFOR (101). Ferredoxin levels, hydrogenosomal malic enzyme, and NAD: ferredoxin oxidoreductase also decrease.

*T. vaginalis* enhances lactate fermentation and glycolysis to compensate. Highly resistant lines have no detectable PFOR activity, PFOR mRNA, or ferredoxin mRNA (25; unpublished data). Alternate 2-oxoacid oxidoreductases have now been described which increase in activity during stepwise selection for increase in drug resistance (25, 216). As in *Giardia*, these alternative oxidoreductases have different substrate requirements, do not necessarily donate electrons to ferredoxin, and appear not to activate metronidazole, thereby circumventing the downregulated PFOR to provide alternative sources of energy. The arginine dihydrolase pathway also provides a source of energy for the parasite (114, 236).

It has been proposed that aerobic drug resistance occurs in infected patients and that the more highly anaerobic resistance can only be generated in the laboratory (101). However, the first proven drug-resistant Australian isolate was highly resistant to metronidazole when also tested anaerobically; therefore, anaerobic resistance can clearly develop in *Trichomonas* infections of humans (224; unpublished data). This is cause for concern, since it has been regarded that anaerobic resistance is unlikely to develop in naturally infected hosts because of the multiple steps that have so far been required to generate anaerobic resistance in the laboratory (101). Two strains have been isolated that are resistant under anaerobic conditions but do not grow aerobically (unpublished data). This is in contrast to previous data which suggest that there is a stepwise progression from aerobic to anaerobic resistance (101) and imply that there is more than one route to resistance. The earlier conflicting data may also be explained by such differences in strains and the differential regulation of metabolic pathways involved in resistance mechanisms.

Although reports of drug-resistant *Trichomonas* have appeared regularly since the phenomenon was first documented in 1962 (118), the occurrences have not increased to epidemic proportions. It has been assumed that resistance may debilitate the parasite and impede its transfer among sexually active partners, leaving the resistance a problem for the carrier alone. Treatment failure is not uncommon (118, 147, 159, 177, 191). In two clinics in the United States (Philadelphia and Detroit), the number of highly resistant cases has been reported as 1 per 2,000 to 3,000 trichomoniasis cases treated prior to 1996 (180). However, this number has increased to 17 in 1997 and 1998, with a clear upward trend, and some cases appear incurable (180). If Lossick's (118) frequency of refractory cases resistant to the highest recommended doses of metronidazole is applied to the 1999 data, 5 to 10% of trichomoniasis isolates now show some level of resistance, e.g., need more than one series of treatments. This is consistent with Centers for Disease Control and Prevention data of 1989 (147). Whether continued drug selection has now allowed increased transmission, more resistant organisms, or the emergence of a more serious sexually transmitted disease problem remains to be monitored.

## CLINICAL AND LABORATORY-GENERATED DRUG-RESISTANT ISOLATES

Laboratory-based studies which have developed systems for inducing drug resistance complement analysis of clinical isolates of *T. vaginalis, G. duodenalis*, and *E. histolytica* (218). Stepwise incremental increases in drug resistance induced by a variety of drug regimens, including increases in drug concentration at sublethal levels or short multiple exposures to lethal levels, that are administered with and without mutagens, have provided considerable information about the ability of parasites to mount a resistance defense and the mechanisms involved (22, 24, 25, 33, 34, 101, 193, 194, 195, 196, 200, 201, 206, 208, 216, 217, 218). With no examples of horizontal, transposon, or plasmid transfer of drug resistance in the anaerobic protozoa, this has been a valid and extremely useful approach to determine mechanisms of resistance. However, a double-stranded RNA virus in *Giardia* (237) and *Trichomonas* (3, 186) and the mini-circular plasmid form of a transposable element in *Giardia* (215) suggest the possibility of transfer. Perhaps the only drawback is the lack of host-parasite interaction in the development of resistance; in many instances, however, any interaction in the maintenance or modulation of resistance could be ascertained later with in vivo studies. Furthermore, mechanisms uncovered in the laboratory can then be readily analyzed for their appearance in clinical isolates; intermediate stages of resistance can be compared for their likelihood to progress to much higher levels. The great advantage of in vitro studies is that the parental, sensitive parasites and the selected, resistant parasites are genetically syngeneic, so that resistance is developed in the same genetic background, and hence confounding or alternate mechanisms do not complicate mechanistic interpretations, a situation which has arisen with malaria, for example (62, 156). A second advantage for in vitro studies with the anaerobic protozoa addressed here is that they have no known sexual stages, so that multifactorial or polygenic resistance traits must arise in the same parasite and can be monitored over time and incremental increases in drug concentration. A third advantage is the ability to determine the resistance capabilities of the parasite before they arise as an epidemiological or public health problem at large; prior knowledge and predictive values of resistance capabilities and mechanisms for malaria, for example, may have alleviated and forestalled the major, and currently insurmountable, resistance problems that there are today (17, 138).

There is only one example of the inability to develop resistance in vitro to an antiparasitic drug, that being development of chloroquine resistance in *Plasmodium falciparum* malaria in two regions, Thailand and South America. Resistance to chloroquine was subsequently transferred or transmitted worldwide (184). Laboratory-induced resistance has not been demonstrated convincingly as yet, although it was readily achieved for *Plasmodium berghei* (150); this is more likely due to a difficulty in screening sufficient numbers of parasites than an inability to induce resistance. A bacterial example of the inability to develop resistance is *Bacteroides fragilis*, which is regarded as a "gold standard" for anaerobic bacterial resistance to metronidazole. Less than 1% resistance has been detected in clinical isolates, and these are not at high drug concentrations (155); it is therefore assumed that resistance to metronidazole in anaerobes is difficult to find or achieve (155). This is clearly not the case for anaerobic protozoa. For example, metronidazole resistance in *Trichomonas* was detected within 1 year of initial drug release. Although resistance may presently be a major problem for the individual harboring such organisms, epidemics of resistant organisms have not been demonstrated so far. Nor is it true for all anaerobic bacteria,

e.g., *Helicobacter pylori* isolates from some parts of the world show high incidences of metronidazole resistance (12).

Such arguments also highlight the history of chloroquine usage and the past lack of resistant *P. falciparum*, for example, but do not reflect the relentless development of resistance to antimicrobials in microorganisms, including *Plasmodium* (156). Although the onset of resistance to multiple drugs in *Plasmodium* has been attributed to sorting of genes conferring different aspects of resistance, alternative interpretations have been proposed; comparing the diversity in different genes, Rich et al. (162) suggest that current isolates examined worldwide may be derived from a very recent (thousands of years) single, clonal propagation and that sexual exchange plays a much less important role than mitotic exchange in generation of diversity. Therefore, even in sexual protozoa, drug resistance may have propagated in a manner similar to that in asexual organisms. This is also consistent with the spread of resistance to chloroquine from two regions (184). Selection under continuous drug pressure also appears to have generated traits (accelerated resistance to multiple drugs) in *Plasmodium* that increase the innate ability to initiate rapid and high resistance to novel compounds that is not the result of sexual gene assortment (156) .

Recently, multiple, simultaneous resistance of *B. fragilis* to metronidazole, coamoxiclav, and imipinem was described for a patient after surgical procedures (198) . Resistance to metronidazole in a series of hospital isolates has been attributed to novel transferable genes (*nimA* to *nimD*) (161) that encode or regulate a metronidazole oxidoreductase (32). *nim* genes have been found in other *B. fragilis* isolates as well as other anaerobes (119). Whether this is the beginning of an increase in resistance mechanisms, as has been observed in malaria for a number of other drugs (156), is of great significance. An example of metroninidazole resistance being clearly on the increase is *Helicobacter pylori*; isolates from countries in which metronidazole can be easily obtained without prescription commonly develop resistance through secondary selection in the gut (12).

Comparison of *Giardia* isolates has shown a range of susceptibilities to metronidazole and furazolidone (208). However, the relevance of point mutations in drug resistance among these isolates is difficult to determine because of considerable genetic diversity; there is up to 30% divergence in the sequence of some genes encoding housekeeping enzymes, and up to 50% for intergenic spacers containing regulatory elements (37, 38, 185, 203, 220, 221). The range of variation in enzymatic activity (unpublished data) also makes interpretation of their role in resistance more difficult. This is further complicated by the presence of mixed genotypes in human infections and differential selection during in vitro culture (207). Major changes, such as chromosomal deletion and complete loss of function, e.g., the elimination of hydrogenosomal enzymes in *Trichomonas*, render correlations much more significant and simplify interpretation. Isogenic strains are therefore essential to follow induced changes and the development of high levels of resistance (218) when there is high genetic diversity. Stepwise induction of resistance in vitro has led to the unusual situation in which strains are more resistant than those so far detected in the wild (101, 218). We are therefore warned in advance of the parasite's resistance capabilities in a number of ways, including resistance mechanisms at the molecular level, common or coordinately regulated pathways of drug activation and drug resistance, the number of enzymatic steps involved, and multiple drug resistance. Knowledge of these resistance mechanisms is complemented by development of ways of circumventing these events, identification of new targets, and development of new drugs (Upcroft et al., unpublished data), and delivery systems which will overcome or bypass them. If novel parasite resistance mechanisms arise in the host, a considerable background knowledge has therefore been generated to determine at which level in the organism this may occur and the relationship to known mechanisms. A framework for a rational approach to drug design and therapy can be envisioned (208, 209, 218; unpublished data).

In a bacterial comparison, the importance of using isogenic strains for analyzing drug resistance and virulence in *Mycobacterium tuberculosis*, another organism which appears not to use plasmid-mediated transfer of resistance determinants, has been emphasized recently (241, 242). Isogenic strains are being used in combination with the natural variation in drug susceptibilities of different *Mycobacterium* species and gene transfer studies to unravel the range of mechanisms now evident in sensitivity and resistance, as well as the participation of subtle pleiotropic enzymatic pathways which augment high levels of resistance (242). The genome sequence of two different strains will also aid in analyzing loss-of-function and recessive mutations involved in the development of resistance (46, 241).

## EPIDEMIOLOGY OF RESISTANCE

Although our understanding of drug resistance in even one bacterium is still incomplete, significant detail about mechanisms such as penicillin resistance and drug transporters at the molecular level has allowed new approaches to be instigated. One of these is the epidemiology and population dynamics of resistance extending beyond the enumeration or prevalence of resistant organisms, which may allow predictive outcomes for the optimized use of current drugs and introduction of new drugs (107, 108). Our intuitive expectation of drug use and subsequent resistance development may not parallel the real capabilities of an organism, e.g., the long-held supposition that the development of resistance is always a burden for the organism and that removal of drug pressure will allow return of sensitive organisms by natural selection is clearly not universally correct (5, 105, 115, 116). In one study of *Salmonella* strains resistant to three antibiotics and thus rendered avirulent, compensatory mutations to virulence occurred more frequently than backmutations to drug sensitivity (16). Similarly, modeling and subsequent experimental testing can provide unexpected outcomes for development of resistance and multidrug resistance that may change the way drugs are rotated in hospital use to prevent the acquisition of resistance and the spread of these strains in the community (8, 14, 69, 107, 108). Such studies are in their infancy for the anaerobic protozoa, but every study of resistant organisms and the mechanisms and spread of resistance adds information to a more complete understanding of the capabilities of these organisms and the dynamics of the host-parasite relationship.

Direct comparison of the anaerobic protozoa with bacteria has been very valuable, particularly in defining the fundamen-

tal metabolic pathways and enzymes involved in activation of drugs and subsequent resistance. This has placed them in an evolutionary position that differs from the aerobic eukaryotes, which has been argued above as being early diverging and distinct from those organisms that now have mitochondria. There are also considerable differences, e.g., detoxification pathways in which SOD, catalase, and peroxidase may be present or absent and unusual organelles in *Trichomonas* and *Entamoeba*. The work with *Giardia* and *Entamoeba* also highlights that each of the protozoa subsequently regulates its essential metabolism, glycolysis, idiosyncratically under drug pressure, e.g., whether PFOR (and ferredoxin) is downregulated and to what extent. As we determine the more subtle aspects of the coupled pathways and differences in bypass and supplementary metabolic systems, such as alternative oxidoreductases, the varieties of ferredoxins and flavodoxins, and detoxification systems, the extent of the diversity in these anaerobes will become even more discernable. Initially grouping them together has been a valuable exercise, but determining these differences will probably add even more to our understanding of the few anaerobic protozoa that have been examined in any real detail. This process will also evoke novel drug targets and drugs for improved control, a challenge for the future.

## ACKNOWLEDGMENTS

We thank the National Health and Medical Research Council of Australia and the Australian Research Council for grant support.

## REFERENCES

1. **Adam, R. D.** 1991. The biology of *Giardia* spp. Microbiol. Rev. **55:**706–732
2. **Aldeen, W. E., K. Carroll, A. Robinson, M. Morrison, and D. Hale.** 1998. Comparison of nine commercially available enzyme-linked immunosorbent assays for detection of *Giardia lamblia* in fecal specimens. J. Clin. Microbiol. **36:**1338–1340.
3. **Alderete, J. F.** 1999. Iron modulates phenotypic variation and phosphorylation of P270 in double-stranded RNA virus-infected *Trichomonas vaginalis*. Infect. Immun. **67:**4298–4302.
4. **Alderete, J. F., M. W. Lehker, and R. Arroyo.** 1995. The mechanisms and molecules involved in cytoadherence and pathogenesis of *Trichomonas vaginalis*. Parasitol. Today **11:**70–74.
5. **Andersson, D. I., and B. R. Levin.** 1999. The biological cost of antibiotic resistance. Curr. Opin. Microbiol. **2:**489–493.
6. **Andrews, R. H., P. T. Monis, P. L. Ey, and G. Mayrhofer.** 1998. Comparison of the levels of intra-specific genetic variation within *Giardia muris* and *Giardia intestinalis*. Int. J. Parasitol. **28:**1179–1185.
7. **Anisoke, J. C., C. O. Onwuliri, R. E. Inyang, J. I. Akoh, B. E. Nwoke, C. M. Adeiyongo, S. N. Okoye, and O. B. Akogun.** 1993. Trichomoniasis amongst students of a higher institution in Nigeria. Appl. Parasitol. **34:**19–25.
8. **Austin, D. J., M. Kakehashi, and R. M. Anderson.** 1997. The transmission dynamics of antibiotic-resistant bacteria: the relationship between resistance in commensal organisms and antibiotic consumption. Proc. R. Soc. Lond. B **264:**1629–1638.
9. **Ayala, P., J. Samuelson, D. Wirth, and E. Orozco.** 1990. *Entamoeba histolytica*: physiology of multidrug resistance. Exp. Parasitol. **71:**169–175.
10. **Báez-Camargo, M., E. Flores-Soto, and E. Orozco.** 1992. Improved molecular karyotype of *Entamoeba histolytica*. Arch. Med. Res. **23:**7–9.
11. **Báez-Camargo, M., A.M. Riverón, D.M. Delgadillo, E. Flores, T. Sánchez, G. Garcia-Rivera, and E. Orozco.** 1996. *Entamoeba histolytica*: gene linkage groups and relevant features of its karyotype. Mol. Gen. Genet. **253:**289–296.
12. **Banatvala, N., G. R. Davies, Y. Abdi, L. Clements, D. S. Rampton, J. M. Hardie, and R. A. Feldman.** 1994. High prevalence of *Helicobacter pylori* metronidazole resistance in migrants to east London: relation with previous nitroimidazole exposure and gastroduodenal disease. Gut **35:**1562–1566.
13. **Band, R. N., and H. Cirrito.** 1979. Growth response of axenic *Entamoeba histolytica* to hydrogen, carbon dioxide, and oxygen. J. Protozool. **26:**282–286.
14. **Baquero, F., M. C. Negri, M. I. Morosini, and J. Blázquez.** 1997. The antibiotic selective process: concentration-specific amplification of low-level resistant populations. Ciba Found. Symp. **207:**93–111.
15. **Biagini, G. A., B. J. Finlay, and D. Lloyd.** 1997. Evolution of the hydrogenosome. FEMS Microbiol. Lett. **155:**133–140.
16. **Björkman, J., D. Hughes, and D. I. Andersson.** 1998. Virulence of antibiotic-resistant *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **95:**3949–3953.
17. **Bloland, P. B., and M. Ettling.** 1999. Making malaria-treatment policy in the face of drug resistance. Ann. Trop. Med. Parasitol. **93:**5–23.
18. **Boreham, P. F. L.** 1994. The current status of chemotherapy for giardiasis, p. 317–328. *In* R. C. A. Thompson, J. A. Reynoldson, and A. J. Lymbery (ed.), *Giardia*: from molecules to disease. CAB International, Cambridge, U.K.
19. **Boreham, P. F. L., R. E. Phillips, and R. W. Shepherd.** 1988. Altered uptake of metronidazole in vitro by stocks of *Giardia intestinalis* with different drug sensitivities. Trans. R. Soc. Trop. Med. Hyg. **82:**104–106.
20. **Borst, P., and M. Ouellette.** 1995. New mechanisms of drug resistance in parasitic protozoa. Annu. Rev. Microbiol. **49:**427–460.
21. **Brasseur, P., and L. Favennec.** 1995. Two cases of giardiasis unsuccessfully treated by albendazole. Parasite **2:**422.
22. **Brown, D. M., J. A. Upcroft, M. R. Edwards, and P. Upcroft.** 1998. Anaerobic bacterial metabolism in the ancient eukaryote *Giardia duodenalis*. Int. J. Parasitol. **28:**149–164.
23. **Brown, D. M., J. A. Upcroft, and P. Upcroft.** 1995. Free radical detoxification in *Giardia duodenalis*. Mol. Biochem. Parasitol. **72:**47–56.
24. **Brown, D. M., J. A. Upcroft, and P. Upcroft.** 1996. A $H_2O$-producing NADH oxidase from the protozoan parasite *Giardia duodenalis*. Eur. J. Biochem. **241:**155–161.
25. **Brown, D. M., J. A. Upcroft, H. N. Dodd, N. Chen, and P. Upcroft.** 1999. Alternative 2-ketoacid oxidoreductase activities in *Trichomonas vaginalis*. Mol. Biochem. Parasitol. **98:**203–214.
26. **Bruchhaus, I., and E. Tannich.** 1994. Purification and molecular characterization of the NAD(+)-dependent acetaldehyde/alcohol dehydrogenase from *Entamoeba histolytica*. Biochem. J. **303:**743–748.
27. **Bult, C. J.,** et al. 1996. Complete genome sequence of the methanogenic archaeon *Methanococcus jannaschii*. Science **273:**1017–1140.
28. **Burchard, G. D.** 1994. Therapy for malaria and amoebiasis. Immun. Infekt. **22:**45–47.
29. **Buret, A.** 1994. Pathogenesis—how does *Giardia* cause disease?, p. 293–315. *In* R. C. A. Thompson, J. A. Reynoldson, and A. J. Lymbery (ed.), *Giardia*: from molecules to disease. CAB International, Cambridge, U.K.
30. **Burgess, D. E.** 1998. Trichomonads and intestinal flagellates, p. 203–214. *In* L. Collier, A. Balows, and M. Sussman (ed.), Topley and Wilson's microbiology and microbial infections, 9th ed, vol. 5: parasitology. Arnold, London, U.K.
31. **Burtin, P., A. Taddio, O. Ariburnu, T. R. Einarson, and G. Koren.** 1995. Safety of metronidazole in pregnancy: a meta-analysis. Am. J. Obstet. Gynecol. **172:**525–529.
32. **Carlier, J.-P., N. Sellier, M.-N. Rager, and G. Reysset.** 1997. Metabolism of a 5-nitroimidazole in susceptible and resistant isogenic strains of *Bacteroides fragilis*. Antimicrob. Agents Chemother. **41:**1495–1499.
33. **Čerkasovová, A., J. Čerkasov, and J. Kulda.** 1988. Resistance of trichomonads to metronidazole. Acta Univ. Carol. Biol. **30:**485–503.
34. **Čerkasovová, A., J. Novák, J. Čerkasov, J. Kulda, and J. Tachezy.** 1988. Metabolic properties of *Trichomonas vaginalis* resistant to metronidazole under anaerobic conditions. Acta Univ. Carol. Biol. **30:**505–512.
35. **Chapman, A., D. J. Linstead, and D. Lloyd.** 1999. Hydrogen peroxide is a product of oxygen consumption by *Trichomonas vaginalis*. J. Biosci. **24:**339–344.
36. **Chen, J., X. Huang, Y. Liu, G. Dai, and W. Chen.** 1996. Detoxicating enzymes of *Entamoeba histolytica* and their detoxifying roles. Chin. Med. J. (Engl. Ed.) **109:**792–794.
37. **Chen, N., J. A. Upcroft, and P. Upcroft.** 1995. A *Giardia duodenalis* gene encoding multiple repeats of a toxin homologue. Parasitology **111:**423–431.
38. **Chen, N., J. A. Upcroft, and P. Upcroft.** 1996. A new cysteine-rich protein-encoding gene family in *Giardia*. Gene **169:**33–38.
39. **Cheney, C. P., and R. K. H. Wong.** 1993. Acute infectious diarrhea. Med. Clin. N. Am. **77:**1169–1196.
40. **Corbeil, L. B.** 1994. Vaccination strategies against *Tritrichomonas foetus*. Parasitol. Today **10:**103–106.
41. **Corsaro, D., D. Venditti, M. Padula, and M. Valassina.** 1999. Intracellular life. Crit. Rev. Microbiol. **25:**39–79.
42. **Cotch, M. F., J. G. Pastorek II, R. P. Nugent, S. L. Hillier, R. S. Gibbs, D. H. Martin, D. A. Eschenbach, R. Edelman, J. C. Carey, J. A. Regan, M. A. Krohn, M. A. Klebanoff, A. V. Rao, G. G. Rhoads, and the Vaginal Infections and Prematurity Study Group.** 1997. *Trichomonas vaginalis* associated with low birth weight and preterm delivery. Sex. Transm. Dis. **24:**353–360.
43. **Coustan, D. R.** 1999. Use of metronidazole during pregnancy. Ped. Infect. Dis. J. **18:**79.
44. **Coustan, D. R., and T. R. Mochizuki.** 1998. Metronidazole, p. 297–298. *In* Handbook for prescribing medications during pregnancy, 3rd ed. Lippincott-Raven, Philadelphia, Pa.
45. **Desenclos, J. C., A. Zergabachew, B. Desmoulins, L. Chouteau, G. Desve, and M. Admassu.** 1988. Clinical, microbiological and antibiotic susceptibil-

ity patterns of diarrhoea in Korem, Ethiopia. J. Trop. Med. Hyg. **91:**296–301.
46. **De Smet, K. A. L.** 1997. *Mycobacterium tuberculosis*: beyond genome sequencing. Trends Microbiol. **5:**429–431.
47. **Dhar, S. K., N. R. Choudhury, V. Mittal, A. Bhattacharya, and S. Bhattacharya.** 1996. Replication initiates at multiple dispersed sites in the ribosomal DNA plasmid of the protozoan parasite *Entamoeba histolytica*. Mol. Cell. Biol. **16:**2314–2324.
48. **Diamond, L. S., and C. G. Clark.** 1993. A redescription of *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911) separating it from *Entamoeba dispar* Brumpt, 1925. J. Eukaryot. Microbiol. **40:**340–344.
49. **Drmota, T., and J. Král.** 1997. Karyotype of *Trichomonas vaginalis*. Eur. J. Protistol. **33:**131–135.
50. **Edwards, M. R., F. V. Gilroy, B. M. Jiminez, and W. J. O'Sullivan.** 1989. Alanine is a major end product of metabolism by *Giardia lamblia*: a proton nuclear magnetic resonance study. Mol. Biochem. Parasitol. **37:**19–26.
51. **Ellis, J. E., D. Cole, and D. Lloyd.** 1992. Influence of oxygen on the fermentative metabolism of metronidazole-sensitive and resistant strains of *Trichomonas vaginalis*. Mol. Biochem. Parasitol. **56:**79–88.
52. **Ellis, J. E., J. M. Wingfield, D. Cole, P. F. L. Boreham, and D. Lloyd.** 1992. Oxygen affinities of metronidazole-resistant and -sensitive stocks of *Giardia intestinalis*. Int. J. Parasitol. **23:**35–39.
53. **Engbring, J. A., J. L. O'Brien, and J. F. Alderete.** 1996. *Trichomonas vaginalis* adhesin proteins display molecular mimicry to metabolic enzymes, p. 207–223. *In* I. Kahane and I. Ofek (ed.), Towards anti-adhesion therapy for microbial diseases. Plenum Press, New York, N.Y.
54. **Erlandsen, S. L., and E. A. Meyer.** 1984. *Giardia* and giardiasis: biology, pathogenesis, and epidemiology. Plenum Press, New York, N.Y.
55. **Espinosa-Cantellano, M., A. González-Robles, B. Chávez, G. Castañón, C. Argüello, A. Lázaro-Haller, and Martínez-Palomo.** 1998. *Entamoeba dispar*: ultrastructure, surface properties and cytopathic effect. J. Eukaryot. Microbiol. **45:**265–272.
56. **Estambale, B. B. A., and R. Knight.** 1992. Protozoan infections and HIV-1 infection: a review. East Afr. Med. J. **69:**373–377.
57. **Fahey, R. C., G. L. Newton, B. Arrick, T. Overdank-Bogart, and S. B. Aley.** 1984. *Entamoeba histolytica*: a eukaryote without glutathione metabolism. Science **224:**70–72.
58. **Fairlamb, A. H., and A. Cerami.** 1992. Metabolism and functions of trypanothione in the Kinetoplastida. Annu. Rev. Microbiol. **46:**695–729.
59. **Farthing, M. J. G.** 1996. Giardiasis. Gastroenterol. Clin. N. Am. **25:**493–515.
60. **Reference deleted.**
61. **Fiori, P. L., P. Rappelli, C. Manca, A. Mattana, and P. Cappuccinelli.** 1992. Phenotypic variation of surface antigenic determinants in *Trichomonas vaginalis* detected by monoclonal antibodies. Microbiologica **15:**227–235.
62. **Foote, S. J., and A. F. Cowman.** 1994. The mode of action and the mechanism of resistance to antimalarial drugs. Acta Trop. **56:**157–171.
63. **Fraser, D., R. Dagan, L. Naggan, V. Greene, J. el-On, Y. Abu-Rbiah, and R. J. Deckelbaum.** 1997. Natural history of *Giardia lamblia* and *Cryptosporidium* infections in a cohort of Israeli Bedouin infants: a study of a population in transition. Am. J. Trop. Med. Hyg. **57:**544–549.
64. **Freeman, C. D., N. E. Klutman, and K. C. Lamp.** 1997. Metronidazole: a therapeutic review and update. Drugs **54:**679–708.
65. **Garcia, L. S.** 1998. Giardiasis, p. 193–202. *In* L. Collier, A. Balows, and M. Sussman (ed.) , Topley and Wilson' s microbiology and microbial infections, 9th ed, vol. 5: parasitology. Arnold, London, U.K.
66. **Garduño-Espinosa, J., M. C. Martinez-Garcia, A. Valadez-Salazar, G. Padilla, R. Cedillo-Rivera, and O. Muñoz.** 1997. Cost-effectiveness analysis of treatment of *E. histolytica*/*E. dispar* cyst carriers. Arch. Med. Res. **28**(Spec. No.)**:**293–294.
67. **Gatti, S., C. Cevini, A. Bruno, S. Novati, and M. Scaglia.** 1995. Transmission of *Entamoeba histolytica* within a family complex. Trans. R. Soc. Trop. Med. Hyg. **89:403–405.**
68. **Gault, M. J., D. S. Reiner, and F. D. Gillin.** 1985. Tolerance of axenically cultured *Entamoeba histolytica* and *Giardia lamblia* to a variety of antimicrobial agents. Trans. R. Soc. Trop. Med. Hyg. **79:**60–62.
69. **Gaynes, R., and D. Monnet.** 1997. The contribution of antibiotic use on the frequency of antibiotic resistance in hospitals. Ciba Found. Symp. **207:**47–60.
70. **Gillin, F. D., and L. S. Diamond.** 1980. *Entamoeba histolytica* and *Entamoeba invadens*: effects of temperature and oxygen tension on growth and survival. Exp. Parasitol. **49:**328–338.
71. **Gillin, F. D., D. S. Reiner, and J. M. McCaffery.** 1996. Cell biology of the primitive eukaryote *Giardia lamblia*. Annu. Rev. Microbiol. **50:**679—705.
72. **Gomez. M. D., D. G. Perez, P. Ayala, J. Samuelson, and E. Orozco.** 1996. Physiology and molecular biology of multidrug resistance in *Entamoeba histolytica*. Arch. Med. Res. **27:**421–425.
73. **Goodwin, A., D. Kersulyte, G. Sisson, S. J. Veldhuyzen van Zanten, D. E. Berg, and P. S. Hoffman.** 1998. Metronidazole resistance in *Helicobacter pylori* is due to null mutations in a gene (*rdxA*) that encodes an oxygen-insensitive NADPH nitroreductase. Mol. Microbiol. **28:**383–393.
74. **Gupta, P. K., and J. K. Frost.** 1990. Cytopathology and histopathology of the female genital tract in *Trichomonas vaginalis* infection, p. 274–290. *In* B. M. Honigberg (ed.), Trichomonads parasitic in humans. Springer-Verlag, New York, N.Y.
75. **Gupta, R. S., and G. B. Golding.** 1996. The origin of the eukaryotic cell. Trends Biochem. Sci. **21:**166–171.
76. **Hager, W. D., and R. P. Rapp.** 1992. Metronidazole. Obstet. Gynecol. Clin. N. Am. **19:**497–510.
77. **Hall, A., and Q. Nahar.** 1993. Albendazole as a treatment for infection with *Giardia duodenalis* in children in Bangladesh. Trans. R. Soc. Trop. Med. Hyg. **87:**84–86.
78. **Haque, R., I. M. Ali, and W. A. Petri, Jr.** 1999. Prevalence and immune response to *Entamoeba histolytica* infection in preschool children in Bangladesh. Am. J. Trop. Med. Hyg. **60:**1031–1034.
79. **Hartman, H. R., and F. A. Kyser.** 1941. Giardiasis and its treatment. J. Am. Med. Assoc. **116:**2835–2839.
80. **Heddi, A., A. M. Grenier, C. Khatchadourian, H. Charles, and P. Nardon.** 1999. Four intracellular genomes direct weevil biology: nuclear, mitochondrial, principal endosymbiont, and *Wolbachia*. Proc. Natl. Acad. Sci. USA **96:**6814–6819
81. **Heyworth, M. F.** 1996. Giardiasis, p. 878–880. *In* D. J. Weatherall, J. G. G. Ledingham, and D. A. Warrell (ed.), Oxford textbook of medicine. Oxford University Press, Oxford, U.K.
82. **Hill, D. R.** 1995. *Giardia lamblia*, p. 2487–2493. *In* G.L. Mandell, J. E. Bennett, and R. Dolin (ed.), Mandell, Douglas and Bennett's principles and practice of infectious diseases. Churchill Livingstone, New York, N.Y.
83. **Hobbs, M. M., P. Kazembe, A. W. Reed, W. C. Miller, E. Nkata, D. Zimba, C. Costello Daly, H. Chakraborty, M. S. Cohen, and I. Hoffman.** 1999. *Trichomonas vaginalis* as a cause of urethritis in Malawian men. Sex. Transm. Dis. **26:**381–387.
84. **Honigberg, B. M.** 1978. Trichomonads of veterinary importance, p. 163–273. *In* J. P. Kreier (ed.), Parasitic protozoa, vol. II. Academic Press, New York, N.Y.
85. **Honigberg, B. M.** 1978. Trichomonads of importance in human medicine, p. 275–454. *In* J. P. Kreier (ed.), Parasitic protozoa, vol. II. Academic Press, New York, N.Y.
86. **Honigberg, B. M.** 1990. Trichomonads found outside the urogenital tract of humans. *In* B. M. Honigberg (ed.), Trichomonads parasitic in humans. Springer-Verlag, New York, N.Y.
87. **Hook, E. W., III.** 1999. Trichomonas vaginalis—no longer a minor STD. Sex. Transm. Dis. **26:**288–389.
88. **Hoosen, A. A., S. M. Ross, M. J. Mulla, and M. Patel.** 1981. The incidence of selected vaginal infections among pregnant urban blacks. S. Afr. Med. J. **59:**827–829.
89. **Humphreys, M., J. Ralphs, L. Durrant, and D. Lloyd.** 1998. Confocal laser scanning microscopy of trichomonads: hydrogenosomes store calcium and show a membrane potential. Eur. J. Protistol. **34:**356–362.
90. **Irusen, E. M., T. F. Jackson, and A. E. Simjee.** 1992. Asymptomatic intestinal colonization by pathogenic Entamoeba histolytica in amebic liver abscess: prevalence, response to therapy, and pathogenic potential. Clin. Infect. Dis. **14:**889–893.
91. **Isaac-Renton, J. L., C. Cordeiro, K. Sarafis, and H. Shahriari.** 1993. Characterization of *Giardia duodenalis* isolates from a waterborne outbreak. J. Infect. Dis. **167:**431–440.
92. **Jarroll, E. L., P. J. Müller, E. A. Meyer, and S. A. Morse.** 1981. Lipid and carbohydrate metabolism of *Giardia lamblia.* Mol. Biochem. Parasitol. **2:**187–196.
93. **Jernigan, J. A., and R. D. Pearson.** 1995. Antiparasitic agents, p. 458–492. *In* G.L. Mandell, J.E. Bennett, and R. Dolin (ed.), Mandell, Douglas and Bennett's principles and practice of infectious diseases. Churchill Livingstone, New York, N.Y.
94. **Johnson, P. J.** 1993. Metronidazole and drug resistance. Parasitol. Today **9:**183–186.
95. **Johnson, P. J., C. J. Lahti, and P. J. Bradley.** 1993. Biogenesis of the hydrogenosome in the anaerobic protist *Trichomonas vaginalis*. J. Parasitol. **79:**664–670.
96. **Klausner, J. D., J. T. Baer, K. M. Contento, and G. Bolan.** 1999. Investigation of a suspected outbreak of vaginal trichomoniasis among female inmates. Sex. Transm. Dis. **26:**335–338.
97. **Knight, R.** 1980. The chemotherapy of amoebiasis. J. Antimicrob. Chemother. **6:**577–593.
98. **Knodler, L. A., E. O. Sekyere, T. S. Stewart, P. J. Schofield, and M. R. Edwards.** 1998. Cloning and expression of a prokaryotic enzyme, arginine deiminase, from a primitive eukaryote *Giardia intestinalis*. J. Biol. Chem. **273:**4470–4477.
99. **Kollaritsch, H., E. Jeschko, and G. Wiedermann.** 1993. Albendazole is highly effective against cutaneous larva migrans but not against *Giardia* infection: results of an open pilot trial in travellers returning from the tropics. Trans. R. Soc. Trop. Med. Hyg. **87:**689.
100. **Krieger, J. N.** 1990. Epidemiology and clinical manifestations of urogenital trichomoniasis in men, p. 235–245. *In* B. M. Honigberg (ed.), Trichomonads parasitic in humans. Springer-Verlag, New York, N.Y.

101. **Kulda, J.** 1999. Trichomonads, hydrogenosomes and drug resistance. Int. J. Parasitol. **29:**199–212.
102. **Reference deleted.**
103. **Kulda, J., and E. Nohýnková.** 1978. Flagellates of the human intestine and of intestines of other species, p. 1–138. *In* J. P. Kreier (ed.), Parasitic protozoa, vol. II. Academic Press, New York, N.Y.
104. **Lehker, M. W., and D. Sweeney.** 1999. Trichomonad invasion of the mucous layer requires adhesins, mucinases, and motility. Sex. Transm. Infect. **75:** 231–238.
105. **Lenski, R. E.** 1997. The cost of antibiotic resistance—from the perspective of a bacterium. Ciba Found. Symp. **207:**131–151.
106. **Leroy, A., M. Mareel, G. De Bruyne, G. Bailey, and H. Nelis.** 1994–1995. Metastasis of *Entamoeba histolytica* compared to colon cancer: one more step in invasion. Invasion Metastasis **14:**177–191.
107. **Levin, B. R., M. Lipsitch, and S. Bonhoeffer.** 1999. Population biology, evolution, and infectious disease: convergence and synthesis. Science **283:** 806–809.
108. **Levin, B. R., M. Lipsitch, V. Perrot, S. Schrag, R. Antia, L. Simonsen, N. Moore Walker, and F. M. Stewart.** 1997. The population genetics of antibiotic resistance. Clin. Infect. Dis. **24**(Suppl. 1)**:**S9–S16.
109. **Li, E., and S. L. Stanley, Jr.** 1996. Amebiasis. Gastroenterol. Clin. N. Am. **25:**471–492.
110. **Lindmark, D. G.** 1980. Energy metabolism of the anaerobic protozoon *Giardia lamblia.* Mol. Biochem. Parasitol. **1:**1–12.
111. **Lindquist, H. D.** 1996. Induction of albendazole resistance in *Giardia lamblia*. Microb. Drug Resist. **2:**433–434.
112. **Lindquist, H. D., A. P. Dufour, L. J. Wymer, and F. W. Schaefer III.** 1998. Criteria for evaluation of proposed protozoan detection. J. Microbiol. Methods **37:**33–43.
113. **Linstead, D. J., and S. Bradley.** 1988. The purification and properties of two soluble reduced nicotinamide:acceptor oxidoreductases from Trichomonas vaginalis. Mol. Biochem. Parasitol. **27:**125–134.
114. **Linstead, D., and M. A. Cranshaw.** 1983. The pathway of arginine catabolism in the parasitic flagellate *Trichomonas vaginalis*. Mol. Biochem. Parasitol. **8:**241–252.
115. **Lipsitch, M., and B. R. Levin.** 1997. The population dynamics of antimicrobial chemotherapy. Antimicrob. Agents Chemother. **41:**363–373.
116. **Lipsitch, M., and B. R. Levin.** 1997. The within-host population dynamics of antibacterial chemotherapy: conditions for the evolution of resistance. Ciba Found. Symp. **207:**112–130.
117. **Liu, S. M., D. M. Brown, P. O'Donoghue, P. Upcroft, and J. A. Upcroft.** 2000. Ferredoxin involvement in metronidazole resistance of *Giardia duodenalis*. Mol. Biochem. Parasitol. **108:**137–140.
118. **Lossick, J. G.** 1990. Therapy of urogenital trichomoniasis, p. 324–341. *In* B. M. Honigberg (ed.), Trichomonads parasitic in humans. Springer-Verlag, New York, N.Y.
119. **Lubbe, M. M., K. Stanley, and L. J. Chalkley.** 1999. Prevalence of *nim* genes in anaerobic/facultative anaerobic bacteria isolated in South Africa. FEMS Microbiol. Lett. **172:**79–83.
120. **Lujan, H. D., M. R. Mowatt, and T. E. Nash.** 1997. Mechanisms of *Giardia lamblia* differentiation into cysts. Microbiol. Mol. Biol. Rev. **61:**294–304.
121. **Mai, Z., S. Ghosh, M. Frisardi, B. Rosenthal, R. Rogers, and J. Samuelson.** 1999. Hsp60 is targeted to a cryptic mitochondrion-derived organelle ("crypton") in the microaerophilic protozoan parasite *Entamoeba histolytica*. Mol. Cell. Biol. **19:**2198–2205.
122. **Malandrin, L., H. Huber, and R. Bernander.** 1999. Nucleoid structure and partition in *Methanococcus jannaschii*: an archaeon with multiple copies of the chromosome. Genetics **152:**1315–1323.
123. **Marshall, M. M., D. Naumovitz, Y. Ortega, and C. R. Sterling.** 1997. Waterborne protozoan pathogens. Clin. Microbiol. Rev. **10:**67–85.
124. **Martin, W., and M. Müller.** 1998. The hydrogen hypothesis for the first eukaryote. Nature **392:**37–41.
125. **Martínez-Palomo, A., and M. Espinosa Cantellano.** 1998. Intestinal ameobae, p. 157–177. *In* L. Collier, A. Balows, and M. Sussman (ed.), Topley and Wilson's microbiology and microbial infections, 9th ed, vol. **5:** parasitology. Arnold, London, U.K.
126. **Meingassner, J. G., and H. Mieth.** 1976. Cross-resistance of trichomonads to 5-nitroimidazole-derivatives. Experientia **32:**183–184.
127. **Mertens, E.** 1993. ATP versus pyrophosphate: glycolysis revisited in parasitic protists. Parasitol. Today **9:**122–126.
128. **Meysick, K., and G. E. Garber.** 1995. *Trichomonas vaginalis*. Curr. Opin. Infect. Dis. **8:**22–25.
129. **Monis, P. T., and R. H. Andrews.** 1998. Molecular epidemiology: assumptions and limitations of commonly applied methods. Int. J. Parasitol. **28:** 981–987.
130. **Moreira, D., and P. Lopez-Garcia.** 1998. Symbiosis between methanogenic archaea and delta-proteobacteria as the origin of eukaryotes: the syntrophic hypothesis. J. Mol. Evol. **47:**517–530.
131. **Müller, M.** 1988. Energy metabolism of protozoa without mitochondria. Annu. Rev. Microbiol. **42:**465–488.
132. **Müller, M.** 1993. The hydrogenosome. J. Gen. Microbiol. **139:**2879–2889.
133. **Müller, M.** 1998. Enzymes and compartmentation of core energy metabolism of anaerobic protists—a special case in eukaryotic evolution?, p. 108–132. *In* G. H. Coombs, K. Vickerman, M. A. Sleigh, and A. Warren (ed.), Evolutionary relationships among protozoa. Chapman and Hall, London, U.K.
134. **Müller, M., and W. Martin.** 1999. The genome of *Rickettsia prowazekii* and some thoughts on the origin of mitochondria and hydrogenosomes. Bioessays **21:**377–381.
135. **Narcisi, E. M., and W. E. Secor.** 1996. In vitro effect of tinidazole and furazolidone on metronidazole-resistant *Trichomonas vaginalis*. Antimicrob. Agents Chemother. **40:**1121–1125.
136. **Nash, T. E., and P. F. Weller.** 1998. Protozoal intestinal infections and trichomoniasis, p. 1202–1203. *In* A. S. Fauci, E. Braunwald, K. J. Isselbacher, J. D. Wilson, J. B. Martin, D. L. Kasper, S. L. Hauser, and D. L. Longo (ed.), Harrison's principles of internal medicine, 14th ed. McGraw-Hill, New York, N.Y.
137. **Neale, K. A., and J. F. Alderete.** 1990. Analysis of the proteinases of representative *Trichomonas vaginalis* isolates. Infect. Immun. **58:**157–162.
138. **Newton, P., and N. White.** 1999. Malaria: new developments in treatment and prevention. Annu. Rev. Med. **50:**179–192.
139. **Newton-Sanchez, O. A., K. Sturm-Ramirez, J. L. Romero-Zamora, J. I. Santos-Preciado, and J. Samuelson.** 1997. High rate of occult infection with *Entamoeba histolytica* among non-dysenteric Mexican children. Arch. Med. Res. **28**(Spec. No.)**:**311–313.
140. **Nozaki, T., T. Asai, S. Kobayashi, F. Ikegami, M. Noji, K. Sato, and T. Takeuchi.** 1998. Molecular cloning and characterization of the genes encoding two isoforms of cysteine synthase in the enteric protozoan parasite *Entamoeba histolytica*. Mol. Biochem. Parasitol. **97:**33–44.
141. **O'Farrell, N., A. A. Hoosen, A. B. Kharsany, and J. van den Ende.** 1989. Sexually transmitted pathogens in pregnant women in a rural South African community. Genitourin. Med. **65:**276–280.
142. **Ondarza, R. N., A. Iturbe, G. Hurtado, E. Tamayo, M. Ondarza, and E. Hernandez.** 1999. *Entamoeba histolytica*: a eukaryote with trypanothione metabolism instead of glutathione metabolism. Biotechnol. Appl. Biochem. **30:**47–52.
143. **Ortega, Y. R., and R. D. Adam.** 1997. *Giardia*: overview and update. Clin. Infect. Dis. **25:**545–550.
144. **Ortner, S., C. G. Clark, M. Binder, O. Scheiner, G. Wiedermann, and M. Duchêne.** 1997. Molecular biology of the hexokinase isoenzyme pattern that distinguishes pathogenic *Entamoeba histolytica* from nonpathogenic *Entamoeba dispar*. Mol. Biochem. Parasitol. **86:**85–94.
145. **Paget, T. A., M. L. Kelly, E. L. Jarroll, D. G. Lindmark, and D. Lloyd.** 1993. The effects of oxygen on fermentation in the intestinal protozoal parasite *Giardia lamblia*. Mol. Biochem. Parasitol. **57:**65–72.
146. **Paget, T. A., and D. Lloyd.** 1990. *Trichomonas vaginalis* requires traces of oxygen and high concentrations of carbon dioxide for optimal growth. Mol. Biochem. Parasitol. **41:**65–72.
147. **Petrin, D., K. Delgaty, R. Bhatt, and G. Garber.** 1998. Clinical and microbiological aspects of *Trichomonas vaginalis*. Clin. Microbiol. Rev. **11:**300–317.
148. **Petter, R., S. Rozenblatt, D. Schechtman, T. E. Wellems, and D. Mirelman.** 1993. Electrophoretic karyotype and chromosome assignments for a pathogenic and nonpathogenic strain of *Entamoeba histolytica*. Infect. Immun. **61:** 3574–3577.
149. **Piper, J. M., E. F. Mitchell, and W. A. Ray.** 1993. Prenatal use of metronidazole and birth defects: no association. Obstet. Gynecol. **82:**348–352.
150. **Platel, D. F., F. Mangou, and J. Tribouley-Duret.** 1999. Role of glutathione in the detoxification of ferriprotoporphyrin IX in chloroquine resistant *Plasmodium berghei*. Mol. Biochem. Parasitol. **98:**215–223.
151. **Quon, D. V., C. E. d'Oliveira, and P. J. Johnson.** 1992. Reduced transcription of the ferredoxin gene in metronidazole-resistant *Trichomonas vaginalis*. Proc. Natl. Acad. Sci. USA **89:**4402–4406.
152. **Rappelli, P., M. F. Addis, F. Carta, and P. L. Fiori.** 1998. *Mycoplasma hominis* parasitism of *Trichomonas vaginalis*. Lancet **352:**1286.
153. **Rappelli, P., M. F. Addis, F. Carta, and P. L. Fiori.** 1998. *Mycoplasma hominis* parasitism of *Trichomonas vaginalis*. Lancet **352:**2023.
154. **Reference deleted.**
155. **Rasmussen, B. A., K. Bush, and F. P. Tally.** 1997. Antimicrobial resistance in anaerobes. Clin. Infect. Dis. **24**(Suppl. 1)**:**S110–S120
156. **Rathod, P. K., T. McErlean, and P.-C. Lee.** 1997. Variations in frequencies of drug resistance in *Plasmodium falciparum*. Proc. Natl. Acad. Sci. USA **94:** 9389–9393.
157. **Ravdin, J. I., and W. A. Petri, Jr.** 1995. Entamoeba histolytica (amebiasis), p. 2395–2408. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Mandell, Douglas and Bennett's principles and practice of infectious diseases. Churchill Livingstone, New York, N.Y.
158. **Reeves, R. E.** 1984. Metabolism of *Entamoeba histolytica* Schaudinn, 1903. Adv. Parasitol. **23:**105–142.
159. **Rein, M. F.** 1995. *Trichomonas vaginalis*, p. 2493–2497. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Mandell, Douglas and Bennett's principles and practice of infectious diseases. Churchill Livingstone, New York, N.Y.
160. **Rein, M. F., and M. Müller.** 1990. *Trichomonas vaginalis* and trichomoni-

asis, p. 481–492. *In* K. K. Holmes, P.-A. Mårdh, P. F. Sparling, and P. J. Wiesner (ed.), Sexually transmitted diseases. McGraw-Hill, New York, N.Y.
161. **Reysset, G.** 1996. Genetics of 5-nitroimidazole resistance in *Bacteroides* species. Anaerobe **2:**59–69.
162. **Rich, S. M., R. R. Hudson, and F. J. Ayala.** 1997. *Plasmodium falciparum* antigenic diversity: evidence of clonal population structure. Proc. Natl. Acad. Sci. USA **94:**13040–13045.
163. **Roberts-Thomson, I. C., R. F. Anders, and P. S Bhathal.** 1982. Granulomatous hepatitis and cholangitis associated with giardiasis. Gastroenterology **83:**480–483.
164. **Rodríguez, M. A., M. E. Hidalgo, T. Sánchez, and E. Orozco.** 1996. Cloning and characterization of the *Entamoeba histolytica* pyruvate:ferredoxin oxidoreductase gene. Mol. Biochem. Parasitol. **78:**273–277.
165. **Roger, A. J., H. G. Morrison, and M. L. Sogin.** 1999. Primary structure and phylogenetic relationships of a malate dehydrogenase gene from *Giardia lamblia*. J. Mol. Evol. **48:**750–755.
166. **Romero Cabello, R., L. R. Guerrero, M. R. Muñoz Garcia, and A. Geyne Cruz.** 1997. Nitazoxanide for the treatment of intestinal protozoan and helminthic infections in Mexico. Trans. R. Soc. Trop. Med. Hyg. **91:**701–703.
167. **Samarawickrema, N., J. A. Upcroft, D. M. Brown, N. Thammapalerd, and P. Upcroft.** 1997. Superoxide dismutase and pyruvate:ferredoxin oxidoreductase involvement in mechanisms of metronidazole resistance in *Entamoeba histolytica*. J. Antimicrob. Chemother. **40:**833–840.
168. **Sanchez, L. B., T. Hashimoto, and M. Müller.** 1996. Sequence of a malic enzyme gene of *Giardia lamblia*. Mol. Biochem. Parasitol. **82:**145–151.
169. **Sanchez, L. B., H. G. Morrison, M. L. Sogin, and M. Müller.** 1999. Cloning and sequencing of an acetyl-CoA synthetase (ADP-forming) gene from the amitochondriate protist, *Giardia lamblia*. Gene **233:**225–231.
170. **Sargeaunt, P. G.** 1994. *Entamoeba histolytica* and *E. dispar*. Trans. R. Soc. Trop. Med. Hyg. **88:**254–255.
171. **Saurina, G. R., and W. M. McCormack.** 1997. Trichomoniasis in pregnancy. Sex. Transm. Dis. **24:**361–362.
172. **Saxena, S. B., and R. R. Jenkins.** Prevalence of *Trichomonas vaginalis* in men at high risk for sexually transmitted diseases. Sex. Transm. Dis. **18:**138–142.
173. **Schmunis, G. A., and F. J. López Antuñano.** 1998. World-wide importance of parasites, p. 19–38. *In* L. Collier, A. Balows, and M. Sussman (ed.), Topley and Wilson's microbiology and microbial infections, 9th ed, vol. **5:** parasitology. Arnold, London, U.K.
174. **Schwebke, J. R.** 1995. Metronidazole: utilization in the obstetric and gynecologic patient. Sex. Transm. Dis. **22:**370–376.
175. **Schwebke, J. R., T. Aira, N. Jordan, P. E. Jolly, and S. H. Vermund.** 1998. Sexually transmitted diseases in Ulaanbaatar, Mongolia. Int. J. STD AIDS **9:**354–358.
176. **Reference deleted.**
177. **Sherrard, J.** 1999. National guideline for the management of *Trichomonas vaginalis*. Sex. Transm. Infect. **75**(Suppl. 1)**:**S21–S23.
178. **Silva, G., S. Oliveira, C. M. Gomes, I. Pacheco, M. Y. Liu, A. V. Xavier, M. Teixeira, J. LeGall, and C. Rodrigues-Pousada.** 1999. *Desulfovibrio gigas* neelaredoxin—a novel superoxide dismutase integrated in a putative oxygen sensory operon of an anaerobe. Eur. J. Biochem. **259:**235–243.
179. **Soave, R.** 1996. *Cyclospora*: an overview. Clin. Infect. Dis. **23:**429–437.
180. **Sobel, J. D., V. Nagappan, and P. Nyirjesy.** 1999. Metronidazole-resistant vaginal trichomoniasis—an emerging problem. N. Engl. J. Med. **341:**292–293.
181. **Sorvillo, F., and P. Kerndt.** 1998. *Trichomonas vaginalis* and amplification of HIV-1 transmission. Lancet **351:**213–214.
182. **Sotto, A., J. L. Alvarez, B. Garcia, F. Pomar, and A. Cendan.** 1990. Acute hepatic lesion caused by Giardia lamblia. Rev. Esp. Enferm. Dig. **77:**24–28.
183. **Stanley, S. L., Jr.** 1997. Progress towards development of a vaccine for amebiasis. Clin. Microbiol. Rev. **10:**637–649.
184. **Su, X.-Z., L. A. Kirkman, H. Fujioka, and T. E. Wellems.** 1997. Complex polymorphisms in an ~330 kDa protein are linked to chloroquine-resistant P. falciparum in Southeast Asia and Africa. Cell **91:**593–603.
185. **Swarbrick, A., R. L. H. Lim, J. A. Upcroft, and T. S. Stewart.** 1998. Nucleotide variation in the cytidine triphospate synthetase gene of Giardia duodenalis. J. Eukaryot. Microbiol. **44:**531–534.
186. **Tai, J. H., H. M. Su, M. F. Shaio, and C. C. Wang.** 1993. The divergence of *Trichomonas vaginalis* among isolates of *Trichomonas vaginalis*. Exp. Parasitol. **76:**278–286.
187. **Tannich, E., I. Bruchhaus, R. D. Walter, and R. D. Horstmann.** 1991. Pathogenic and nonpathogenic *Entamoeba histolytica*: identification and molecular cloning of an iron-containing superoxide dismutase. Mol. Biochem. Parasitol. **49:**61–71.
188. **Taylor-Robinson, D.** 1998. *Mycoplasma hominis* parasitism of *Trichomonas vaginalis*. Lancet **352:**2022–2023.
189. **Thammapalerd, N., D. Kotimanusvanij, M. Duchêne, J. A. Upcroft, R. Mitchell, A. Healey, N. Samarawickrema, S. Tharavanij, G. Wiedermann, and P. Upcroft.** 1996. Pyruvate:ferredoxin oxidoreductase from *Entamoeba histolytica* recognised by a monoclonal antibody. Southeast Asian J. Trop. Med. Public Health **27:**63–70.
190. **Thompson, R. C. A., J. A. Reynoldson, and A. H. W. Mendis.** 1993. *Giardia* and giardiasis. Adv. Parasitol. **32:**71–160.
191. **Tierney, L. M., Jr., S. J. McPhee, and M. A. Papadakis (ed.).** 1999. Current medical diagnosis and treatment 1999. Appleton & Lange, Stamford, Conn.
192. **Tovar, J., A. Fischer, and C. G. Clark.** 1999. The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*. Mol. Microbiol. **32:**1013–1021.
193. **Townson, S. M., P. F. L. Boreham, P. Upcroft, and J. A. Upcroft.** 1994. Resistance to the nitroheterocyclic drugs. Acta Trop. **56:**173–194.
194. **Townson, S. M., G. R. Hanson, J. A. Upcroft, and P. Upcroft.** 1994. A purified ferredoxin from *Giardia duodenalis*. Eur. J. Biochem. **220:**439–446.
195. **Townson, S. M., H. Laqua, P. Upcroft, P. F. L. Boreham, and J. A. Upcroft.** 1992. Induction of metronidazole and furazolidone resistance in *Giardia*. Trans. R. Soc. Trop. Med. Hyg. **86:**521–522.
196. **Townson, S. M., J. A. Upcroft, and P. Upcroft.** 1996. Characterisation and purification of pyruvate:ferredoxin oxidoreductase from *Giardia duodenalis*. Mol. Biochem. Parasitol. **79:**183–193.
197. **Tseng, C.-P., J. Albrecht, and R. P. Gunsalus.** 1996. Effect of microaerophilic cell growth conditions on expression of the aerobic (*cyoABCDE* and *cydAB*) and anaerobic (*narGHJI*, *frdABCD*, and *dmsABC*) respiratory pathway genes in *Escherichia coli*. J. Bacteriol. **178:**1094–1098.
198. **Turner, P., R. Edwards, V. Weston, A. Gazis, P. Ispahani, and D. Greenwood.** 1995. Simultaneous resistance to metronidazole, co-amoxiclav, and imipinem in clinical isolate of *Bacteroides fragilis*. Lancet **345:**1275–1277.
199. **Upcroft, J. A., P. F. L. Boreham, R. W. Campbell. R. W. Shepherd, and P. Upcroft.** 1995. Biological and genetic analysis of a longitudinal collection of *Giardia* samples derived from humans. Acta Trop. **60:**35–46.
200. **Upcroft, J. A., R. W. Campbell, and P. Upcroft.** 1996. Quinacrine-resistant *Giardia duodenalis*. Parasitology **112:**309–313.
201. **Upcroft, J. A., R. W. Campbell, K. Benakli, P. Upcroft, and P. Vanelle.** 1999. The efficacy of new 5-nitroimidazoles against metronidazole-susceptible and -resistant anaerobic protozoa. Antimicrob. Agents Chemother. **43:** 73–76.
202. **Upcroft, J. A., A. J. Capon, A. Dharmkrong-At, A. Healey, P. F. L. Boreham, and P. Upcroft.** 1987. *Giardia intestinalis* antigens expressed in *Escherichia coli*. Mol. Biochem. Parasitol. **26:**267–276.
203. **Upcroft, J. A., A. Healey, R. W. Mitchell, and P. Upcroft.** 1994. A new rDNA repeat unit in human *Giardia*. J. Eukaryot. Microbiol. **41:**639–642.
204. **Upcroft, J. A., P. A. McDonnell, A. N. Gallagher, N. Chen, and P. Upcroft.** 1997. Lethal *Giardia* from a wild-caught sulphur-crested cockatoo (*Cacatua galerita*) established *in vitro* chronically infects mice. Parasitology **114:**407–412.
205. **Upcroft, J. A., P. A. McDonnell, and P. Upcroft.** 1998. Virulent avian *Giardia duodenalis* pathogenic for mice. Parasitol. Today **14:**281–284.
206. **Upcroft, J. A., R. Mitchell, N. Chen, and P. Upcroft.** 1996. Albendazole resistance in *Giardia* is correlated with cytoskeletal changes but not with a mutation at amino acid 200 in β-tubulin. Microb. Drug Resist. **2:**303–308.
207. **Upcroft, J. A., and P. Upcroft.** 1994. Two distinct varieties of *Giardia* in a mixed infection from a single human patient. J. Eukaryot. Microbiol. **41:** 189–194.
208. **Upcroft, J. A., and P. Upcroft.** 1993. Drug resistance and *Giardia*. Parasitol. Today **9:**187–190.
209. **Upcroft, J. A., and P. Upcroft.** 1993. Metronidazole and drug resistance. Parasitol. Today **9:**417–418.
210. **Reference deleted.**
211. **Reference deleted.**
212. **Reference deleted.**
213. **Reference deleted.**
214. **Reference deleted.**
215. **Upcroft, J. A., and P. Upcroft.** 1998. My favourite cell: *Giardia*. BioEssays **20:**256–263.
216. **Upcroft, J. A., and P. Upcroft.** 1999. Keto-acid oxidoreductases in the anaerobic protozoa. J. Eukaryot. Microbiol. **46:**447–449.
217. **Upcroft, P.** 1994. Multiple drug resistance in the pathogenic protozoa. Acta Trop. **56:**195–212.
218. **Upcroft, P.** 1998. Drug resistance in *Giardia*: clinical versus laboratory isolates. Drug. Resist. Updates **1:**166–168.
219. **Upcroft, P., N. Chen, and J. A. Upcroft.** 1997. Telomeric organization of a variable and inducible toxin gene family in the ancient eukaryote *Giardia duodenalis*. Genome Res. **7:**37–47.
220. **Upcroft, P., and J. A. Upcroft.** 1999. Organization and structure of the *Giardia* genome. Protist **150:**17–23.
221. **Upcroft, P., and J. A. Upcroft.** 1999. Lessons from *Giardia* on genome analysis of protozoan parasites. Int. J. Parasitol. **29:**1389–1391.
222. **Reference deleted.**
223. **Viikki, M., E. Pukkala, P. Nieminen, and M. Hakama.** 2000. Gynaecological infections as risk determinants of subsequent cervical neoplasia. Acta Oncol. **39:**71–75.
224. **Voolmann, T., and P. Boreham.** 1993. Metronidazole resistant *Trichomonas vaginalis* in Brisbane. Med. J. Aust. **159:**490.

225. **Voolman, T., F. Morey, and G. Rich.** 1995. Trichomoniasis is a problem in aboriginal women—fact or fiction? Venereology **8:**34–36.
226. **Wallis, P. M.** 1994. Abiotic transmission—is water really significant?, p. 99–122. *In* R. C. A. Thompson, J. A. Reynoldson, and A. J. Lymbery (ed.), Giardia: from molecules to disease. CAB International, Wallingford, U.K.
227. **Wassmann, C., A. Hellberg, E. Tannich, and I. Bruchhaus.** 1999. Metronidazole resistance in the protozoan parasite *Entamoeba histolytica* is associated with increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase. J. Biol. Chem. **274:**26051–26056.
228. **Reference deleted.**
229. **Weinbach, E. C., C. Elwood-Claggett, D. B. Keister, L. S. Diamond, and H. Kon.** 1980. Respiratory metabolism of *Giardia lamblia*. J. Parasitol. **66:**347–350.
230. **Wilkinson, D., S. S. Abdool Karim, A. Harrison, M. Lurie, M. Colvin, C. Connolly, and A. W. Sturm.** 1999. Unrecognized sexually transmitted infections in rural South African women: a hidden epidemic. Bull. World Health Org. **77:**22–28.
231. **Willhoeft, U., and E. Tannich.** 1999. The electrophoretic karyotype of *Entamoeba histolytica*. Mol. Biochem. Parasitol. **99:**41–53.
232. **Wittner, M., and H. B. Tanowitz.** 1992. Intestinal parasites in returned travellers. Med. Clin. N. Am. **76:**1433–1448.
233. **World Health Organization.** 1998. Intestinal parasites control: Burden and trends. WHO Division of Control of Tropical Diseases, World Health Organization, Geneva, Switzerland.
234. **Wu, G., A. Fiser, B. ter Kuile, A. Sali, and M. Müller.** 1999. Convergent evolution of *Trichomonas vaginalis* lactate dehydrogenase from malate dehydrogenase. Proc. Natl. Acad. Sci. USA **96:**6285–6290.
235. **Yarlett, N., N. C. Yarlett, and D. Lloyd.** 1986. Metronidazole-resistant clinical isolates of *Trichomonas vaginalis* have lowered oxygen affinities. Mol. Biochem. Parasitol. **19:**111–116.
236. **Yarlett, N., M. P. Martinez, M. A. Moharrami, and J. Tachezy.** 1996. The contribution of the arginine dihydrolase pathway to energy metabolism by *Trichomonas vaginalis*. Mol. Biochem. Parasitol. **78:**117–125.
237. **Yu, D. C., A. L. Wang, and C. C. Wang.** 1996. Amplification, expression, and packaging of a foreign gene by giardiavirus in *Giardia lamblia*. J. Virol. **70:**8752–8757.
238. **Yuh, Y.-S., J.-Y. Liu, and M.-F. Shaio.** 1997. Chromosome number of *Trichomonas vaginalis*. J. Parasitol. **83:**551–553.
239. **Yung, A. P., and T. A. Ruff.** 1994. Travel medicine 2: upon return. Med. J. Aust. **160:**206–212.
240. **Zaat, J. O., T. G. Mank, and W. J. Assendelft.** 1997. A systematic review on the treatment of giardiasis. Trop. Med. Int. Health **2:**63–82.
241. **Zhang, Y.** 1996. Life without KatG. Trends Microbiol. **4:**415–416.
242. **Zhang, Y., S. Dhandayuthapani, and V. Deretic.** 1996. Molecular basis for the exquisite sensitivity of *Mycobacterium tuberculosis* to isoniazid. Proc. Natl. Acad. Sci. USA **93:**13212–13216.
243. **Zhang, Y.-Q., Z.-Y. Wang, S.-Q. Lu, and M.-L. Feng.** 1986. A familial infection of giardiasis. Chin. Med. J. (Engl. Ed.) **99:**417–419.
244. **Zhong, H.-L., W.-J. Cao, J. F. Rossignol, M.-L. Feng, R.-Y. Hu, S.-B. Gan, and W. Tan.** 1986. Albendazole in nematode, cestode, trematode and protozoan (*Giardia*) infections. Chin. Med. J. (Engl. Ed.) **99:**912–915.