Protozoans: An Introduction to Eukaryotic Pathogens in Man

Introduction

There are other micro-organisms that are pathogenic to man besides bacteria. Some examples of these other groups include fungi, tape worms, round worms, flukes and viruses, to name a few. Another group that was not listed is the protozoans. In general, the study of parasites, parasitology, may be crudely summarized in **Table 1** as follows:

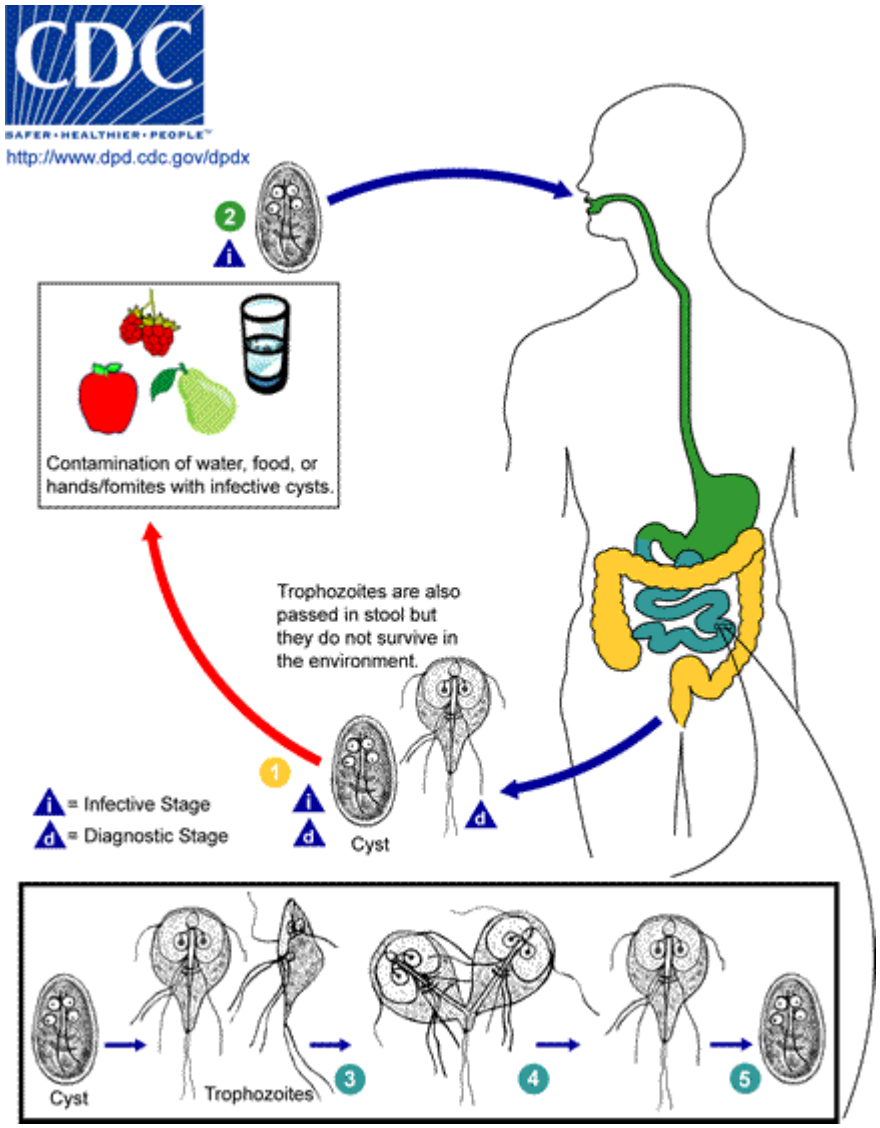
<table>
<tr><td colspan="6">Parasitology</td></tr>
<tr><td colspan="2">Platyhelminthes</td><td>Nemathelminthes</td><td colspan="3">Protozoa</td></tr>
<tr><td colspan="2">Flat worms</td><td>Round worms</td><td>Mastigophora</td><td>Sarcodina</td><td>Sporozoans</td></tr>
<tr><td>Cestodes</td><td>Trematodes</td><td>Nematodes</td><td>Flagellates</td><td>Ameboid</td><td>Sexual and asexual reproduction with 2 different hosts</td></tr>
<tr><td>Tape worms</td><td>Flukes</td><td>Giant round worms</td><td>Giardia, Trichomonas</td><td>Entamoeba, Endolimax</td><td>Plasmodia</td></tr>
</table>

Table 1. Crude summary of parasitology.

While **Table 1** is not all inclusive, it nevertheless serves its purpose as an introduction into this experiment and the following experiment -- both of which involve some aspect of parasitology.

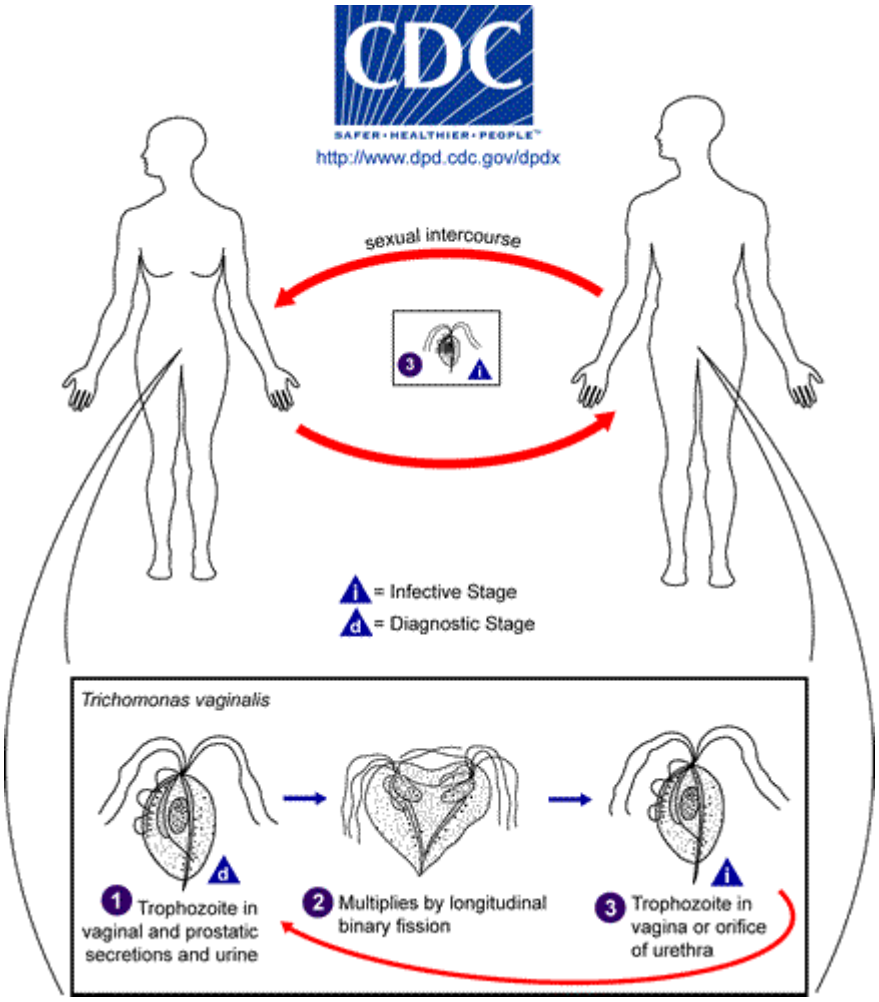
For this specific experiment, each student will observe the following protozoans: *Giardia lamblia, Trichomonas vaginalis, Plasmodium vivax, Plasmodium ovale (difficult to tell from P. vivax), Plasmodium malariae* and *Plasmodium falciparum*. Each protozoan has its own physical characteristic[s] which makes it easy to identify under the microscope. In order to assist the student in identifying the first two protozoans above, they are presented in life cycle format complete with micrographic representations and with citation from the CDC's NCID, below.

Giardia lamblia



http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Giardiasis_il.htm

Trichomonas vaginalis



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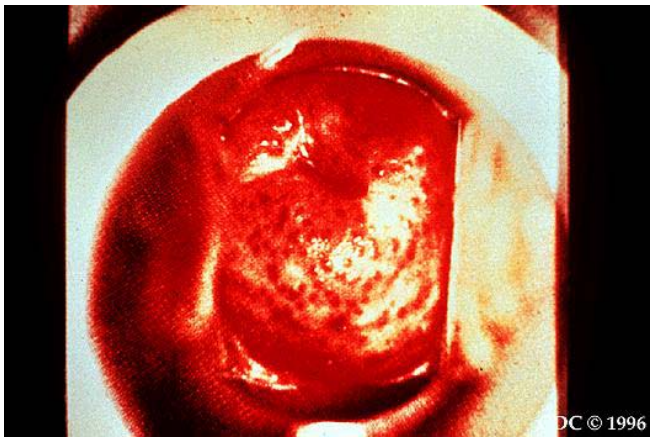


The above graphic is also from the CDC and illustrates the "strawberry cervix" in trichomoniasis. T. vaginalis is specific to the human and is an STD.

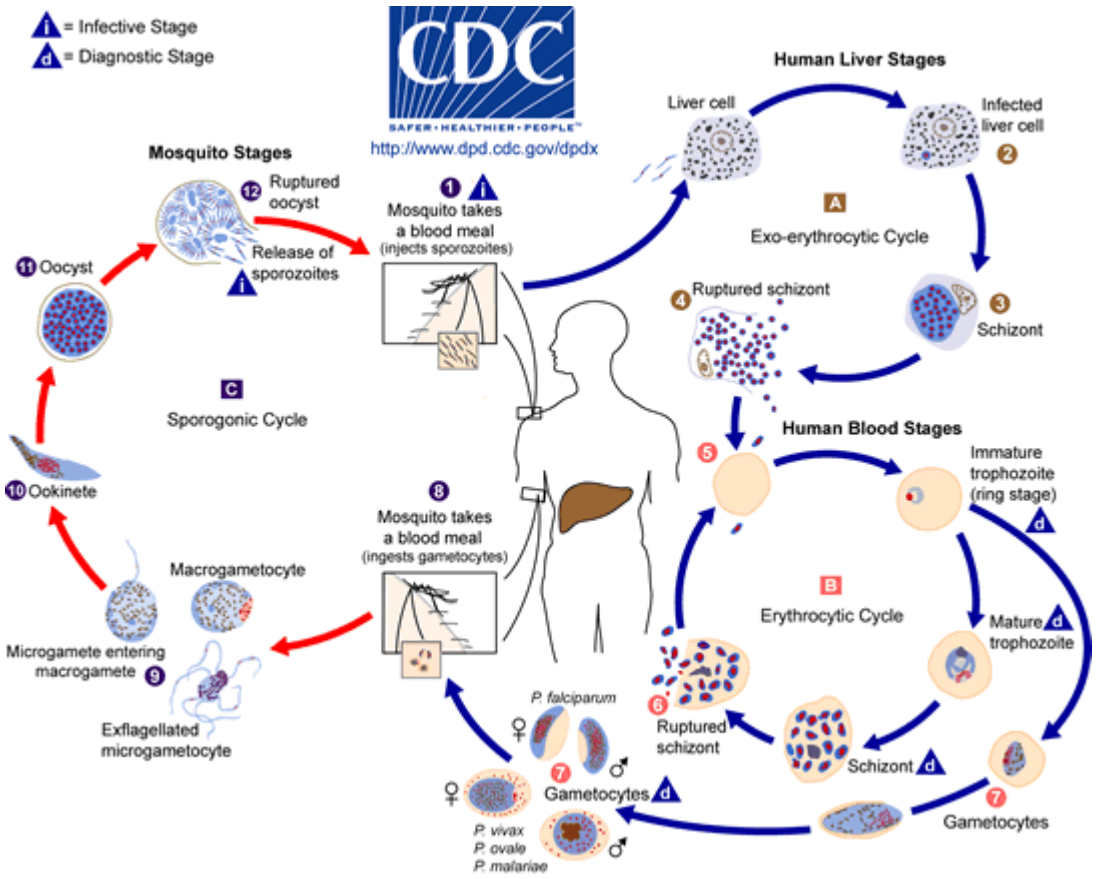
Until recently, *Pneumocystis carinii* was classified as a protozoan and is included in this section of study purely for historical reasons. *P. carinii* has now been classified as a fungus based on its RNA. *P. carinii* is perhaps best known as the causative agent of a pneumonia known as **PCP** (*Pneumocystis carinii* pneumonia) in patients with AIDS. Various sources report the description of *P. carinii* ranging from rosettes of 8 cysts to cysts arranged in any manner "they so choose".

Table 2 below summarizes the more common aspects of the diseases produced by these three micro-organisms. One extra is included for historical purposes -- old and recent. The first three diseases are diagnosed from examining fresh stool specimens microscopically and the last is through microscopic examination of a proper sputum sample or of a sample obtained by bronchial washing.

Micro-organism	Effects
G. lamblia	Giardiasis -- usually only weakly pathogenic for humans; with some immune challenge, get diarrhea: watery, semisolid, greasy, bulky, foul smelling throughout infection; children more sensitive than adults.
T. vaginalis	Trichomoniasis -- : infection limited to vulva, vagina and cervix; mucosa tender, inflamed, eroded; covered with frothy yellow to cream-colored discharge; local tenderness, vulval pruritis and burning. : prostate, seminal vesicles and urethra may be infected; about 10% have thin white urethral discharge.
E. histolytica	"Amoebic dysentery" -- pathological changes ALWAYS due to trophozoites; ulceration of the cecum; extreme abdominal tenderness, dysentery, dehydration, cramps, loss of appetite, weight loss; most common protozoal infection in gay men.
P. carinii	*Pneumocystis carinii pneumonia* -- infantile: alveoli filled with organisms and foamy material; with immunocompromisation comes a febrile pneumonitis with cyanosis: responsible for > 50% of AIDS deaths

Table 2. Summary of diseases.

The last of the protozoans for study in this experiment are of the genus *Plasmodium*. This is the genus of protozoans that causes malaria. There are four species of *Plasmodium*: *ovale, vivax, malariae* and *falciparum*. The *Plasmodia* are interesting from the perspective that they have two distinctly different portions of their life cycles: one in the mosquito (Sporogony) and one in the human [host] (Schizogony). It is because of these phases that the micro-organism, 1) may be visualized and 2) identified in blood smears. A general CDC illustration for the life cycle of Plasmodia spp. is below:



http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Malaria_il.htm

The exogenous phase, or sporogony, occurs in the mosquito. Human blood containing the reproductive cells of the *Plasmodia* (female = macrogametocyte; male = microgametocyte) is taken up when the mosquito bites the human. The reproductive cells are fertilized and develop into sporozoites in the outer layer of the stomach wall. The sporozoites then pass into the mosquito's salivary glands. When the mosquito bites her next victim, the sporozoites are injected into the human along with an anticoagulant (this

makes it easy for the mosquito to aspirate the blood from the human). Thus ends sporogony.

The end of sporogony signals the beginning of schizogony, or the endogenous phase, of the life cycle of *Plasmodia*. The endogenous phase occurs in the human. As the sporozoites are released into the blood of the human, they travel to the liver. The sporozoites multiply and differentiate in the liver and become merozoites. When the merozoites are mature, they enter the blood and invade red blood cells. The merozoites then differentiate into trophozoites and schizonts, respectively. When the schizonts become mature, the red blood cell lyses and more merozoites are released into the blood to begin this whole erythrocytic cycle all over, again. Some of the trophozoites, however, rather than differentiating into schizonts, are released from the red blood cells where they differentiate into the reproductive cells mentioned above. At this point, if a mosquito bites a human, this signals the end of schizogony and re-initiates sporogony.

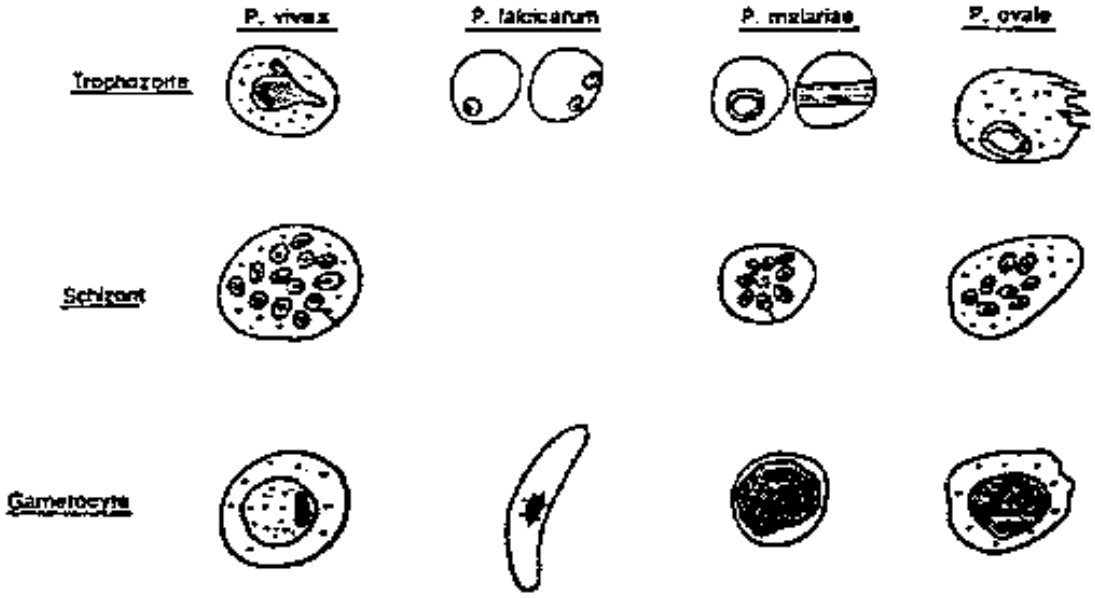
With the release of the merozoites from red blood cell lysis (at 72 hours for *malariae* and 48 hours for the other three species) comes chilling, nausea, vomiting and headache. The second phase of the process is the febrile phase characterized by a fever which may spike as high as 40°C. The third phase is the sweating phase during which time, the patient begins to feel better. With the infection in its early stages, the three phases (hemolysis, fever, sweat) come irregularly. With advancing disease, the cycle repeats at regular intervals, i.e., 48 - 72 hours.

If the infection is not treated, and if the patient does not die as a result of infection, one may expect *P. falciparum* to spontaneously terminate within a year; *P. ovale* and *P. vivax* spontaneously terminate within 5 years; *P. malariae* has been reported to cause relapses for up to 40 years. The least offensive of the four *Plasmodia* are *vivax* and *ovale*; *falciparum* will infect red blood cells of any age, all at once (the others won't), and make the disease course more rigorous for the patient.

The identification of *Plasmodium* depends upon making two blood smears: a thin smear and a thick smear. The thin smear is performed just as for making a peripheral blood smear; the thick smear is prepared by dropping one drop of blood on the slide and allowing it to air dry. Usually these smears are made on the same microscope slide. The slides are then stained by the Giemsa method and analyzed for the micro-organisms. The purpose of the

thick smear is to concentrate the critters so that a diagnosis of *Plasmodium* may be made. The purpose of the this smear is to identify what species is present -- it is always possible for multiple types of infections by two or more *Plasmodia* to be present.

In the figure, below, the various important stages of the development of the four *Plasmodia* are sketched. The ring forms for *falciparum* are usually the only forms observed for this species. One difficulty in utilizing this method of diagnosing malaria is that *ovale* and *vivax* tend to look a lot alike. This difficulty is overcome by the similarities in the respective disease states caused by these two species and by the fact that they are treated in the same manner.

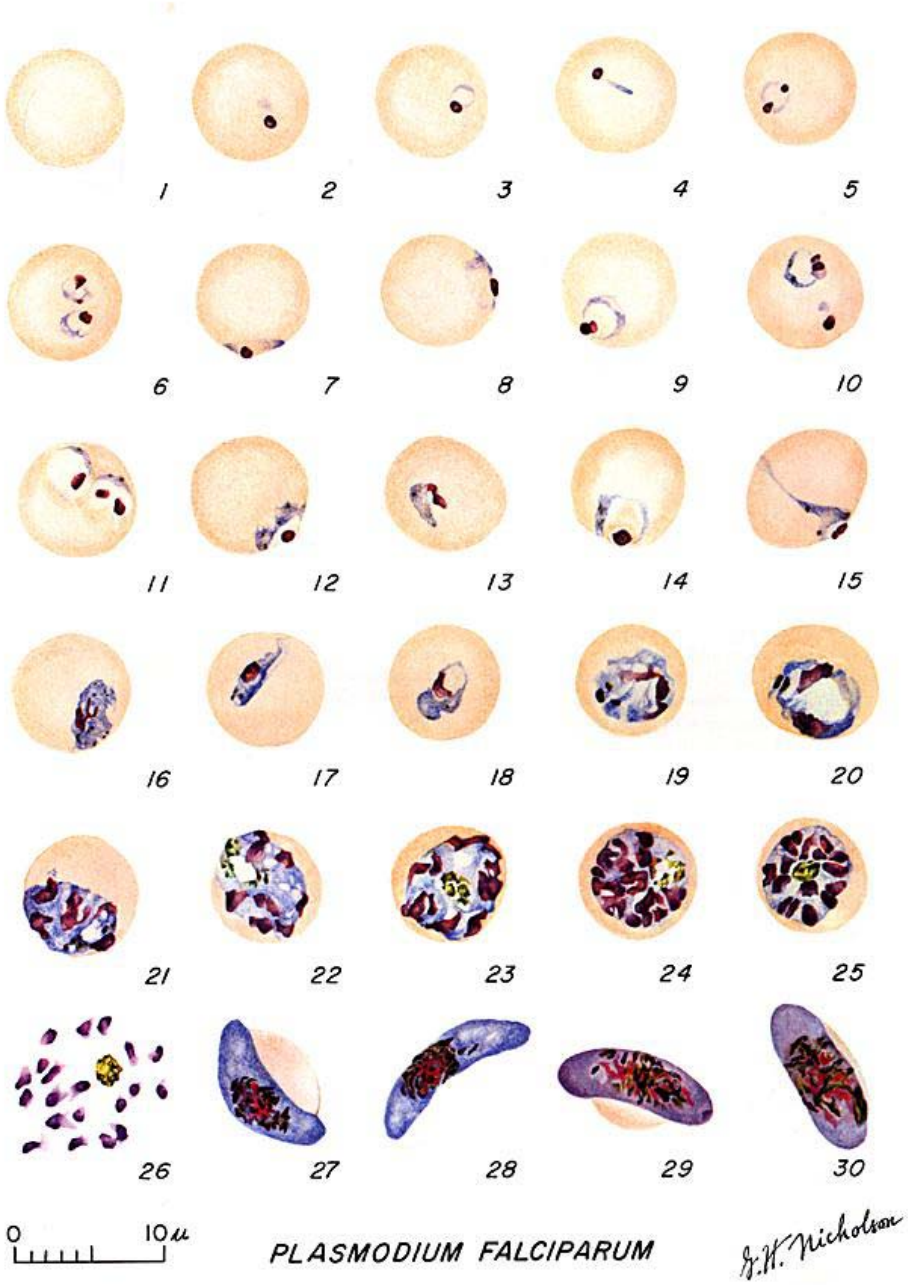


(J. D. MacLean -- http://www.med.mcgill.ca/tropmed/txt/lecture2.htm)

With rare exceptions (and you'll see why shortly), P. falciparum is the only spp. to exhibit "double ring forms"; P. vivax share Schüffner dots. One item to remember is that *Plasmodia* need perfectly spherical red blood cells in order to be invasive. People who have sickle cell anemia do not get malaria because of their inherited trait.

The following images are from the NCID website (http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/) and are cited beneath

the image – the caption that goes with each image is beneath the image, as well.



Illustrations from: Coatney GR, Collins WE, Warren M, Contacos PG. The Primate Malarias. Bethesda: U.S. Department of Health, Education and Welfare; 1971.

Fig. 1: Normal red cell; **Figs. 2**-**18:** Trophozoites (among these, **Figs. 2**-**10** correspond to ring-stage trophozoites); **Figs. 19**-**26:** Schizonts (**Fig. 26** is a ruptured schizont); **Figs. 27**, **28:** Mature macrogametocytes (female); **Figs. 29**, **30:** Mature microgametocytes (male).

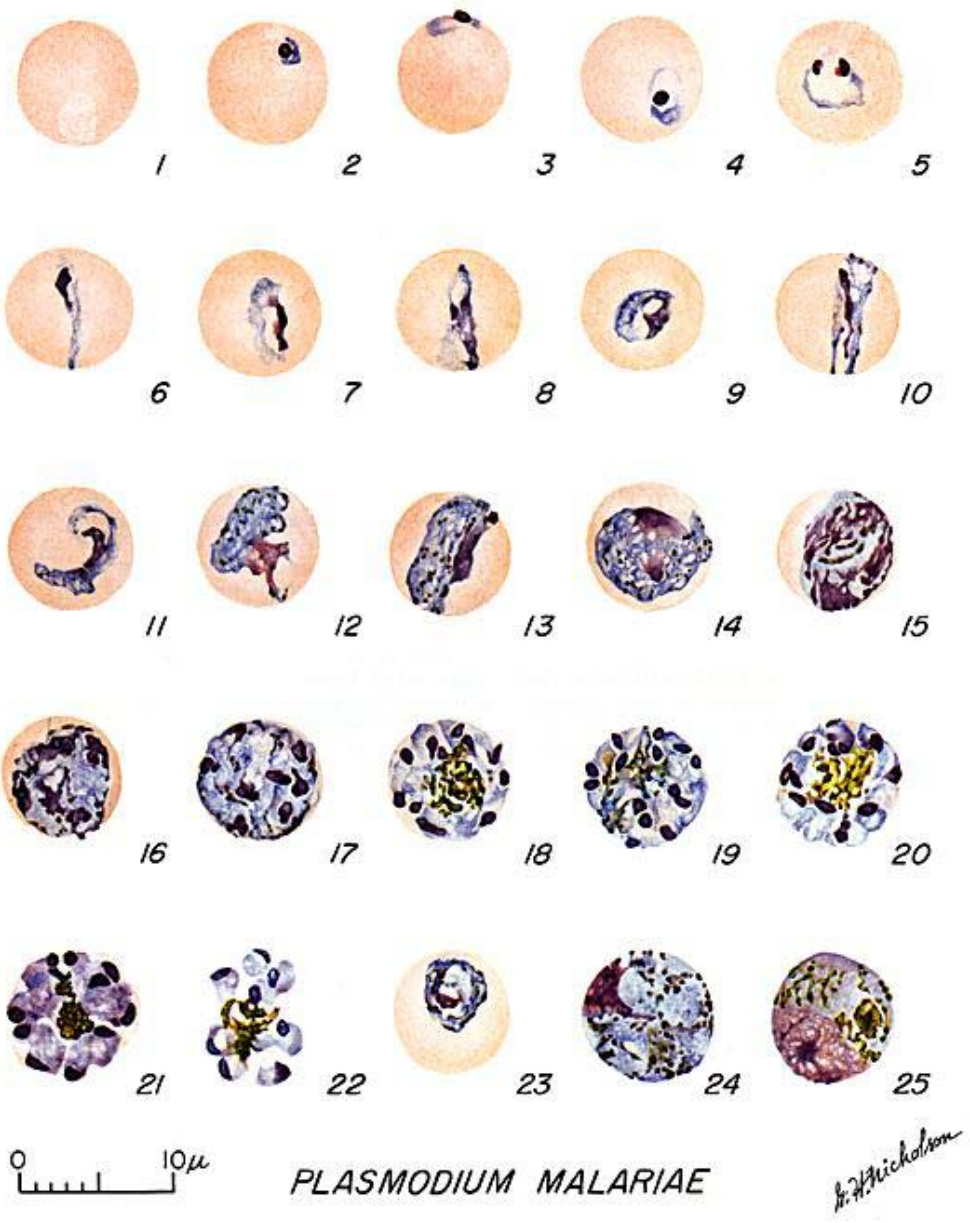


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Fig. 1: Normal red cell; **Figs. 2**-**5:** Young trophozoites (rings); **Figs. 6**-**13:** Trophozoites; **Figs. 14**-**22:** Schizonts; **Fig. 23:** Developing gametocyte; **Fig. 24:** Macrogametocyte (female); **Fig. 25:** Microgametocyte (male).

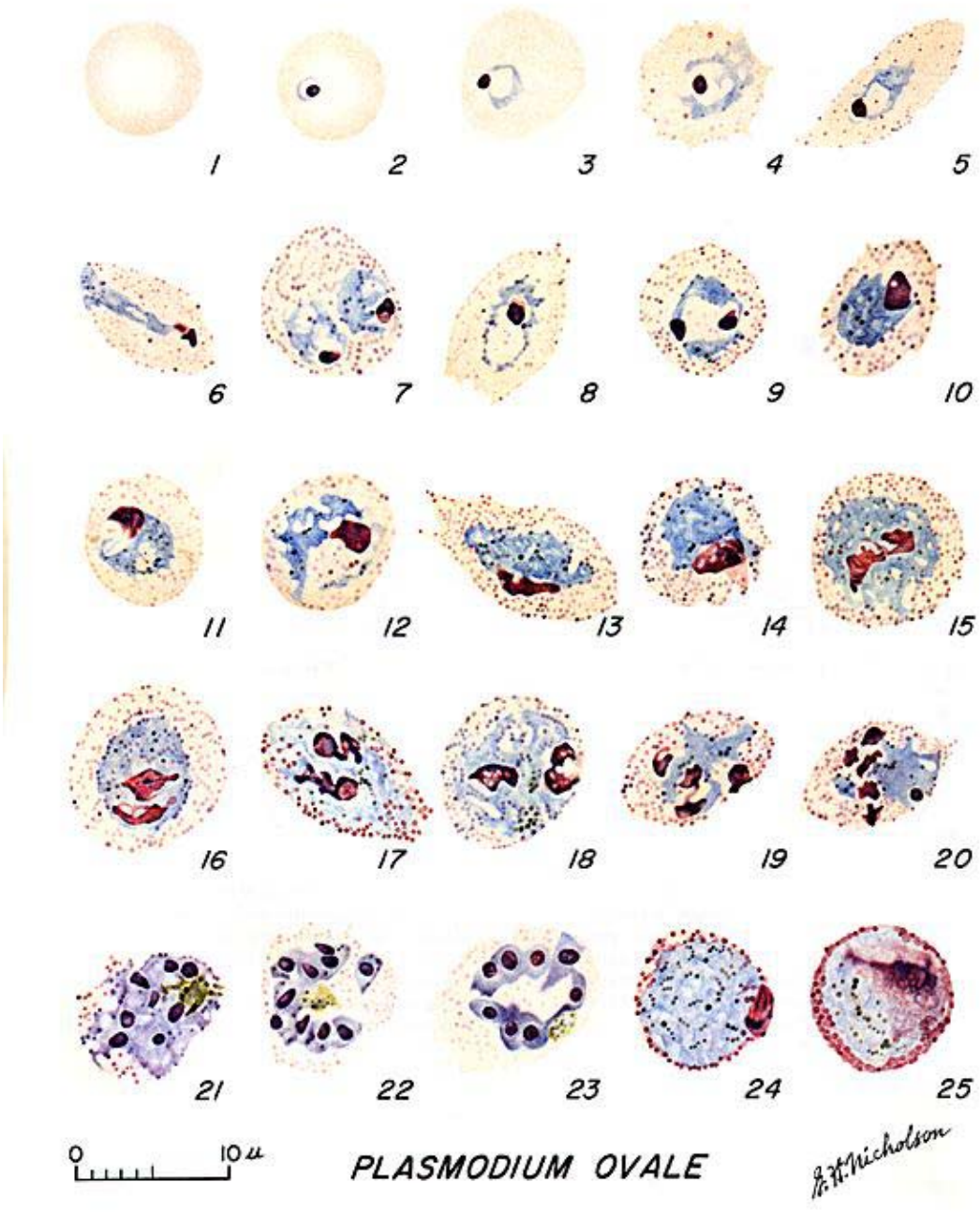

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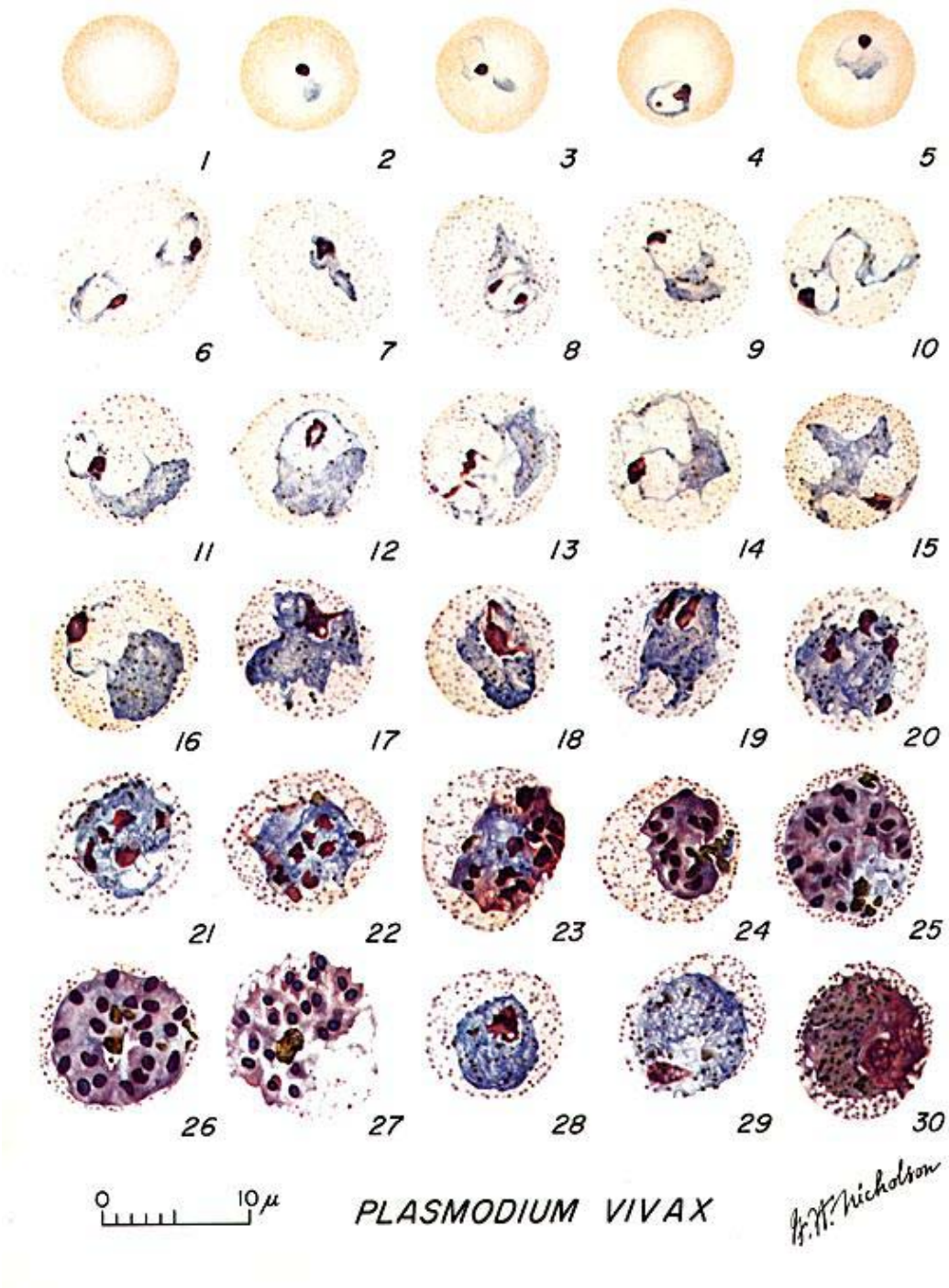
Fig. 1: Normal red cell; **Figs. 2**-**5:** Young trophozoites (Rings); **Figs. 6**-**15:** Trophozoites; **Figs. 16**-**23:** Schizonts; **Fig. 24:** Macrogametocytes (female); **Fig. 25:** Microgametocyte (male).



Illustrations from: Coatney GR, Collins WE, Warren M, Contacos PG. The Primate Malarias. Bethesda: U.S. Department of Health, Education and Welfare; 1971.

Fig. 1: Normal red cell; **Figs. 2**-**6:** Young trophozoites (ring stage parasites); **Figs. 7**-**18:** Trophozoites; **Figs. 19**-**27:** Schizonts; **Figs. 28** and **29:** Macrogametocytes (female); **Fig. 30:** Microgametocyte (male).

Materials and Methods

Materials

Slide of *G. lamblia*	Slide of *T. vaginalis*	Lens paper	Microscope
Slide of *P. malariae*	Slide of *P. vivax*	Slide of *P. falciparum*	Immersion oil

Method

Obtain the slides above and study them under oil immersion. When necessary, ask your instructor for assistance. In the space below, draw what you observe.

G. lamblia	*T. vaginalis*

P. vivax	*P. malaria*	*P. falciparum*

REFERENCES

1.Jawetz, E., Ed., *et al*: **Medical Microbiology, Eighteenth Edition**. (Appleton and Lange: San Mateo) ©1989.

2.Jawetz, E., Ed., *et al*: **Medical Microbiology, Nineteenth Edition**. (Appleton and Lange: San Mateo) ©1991.