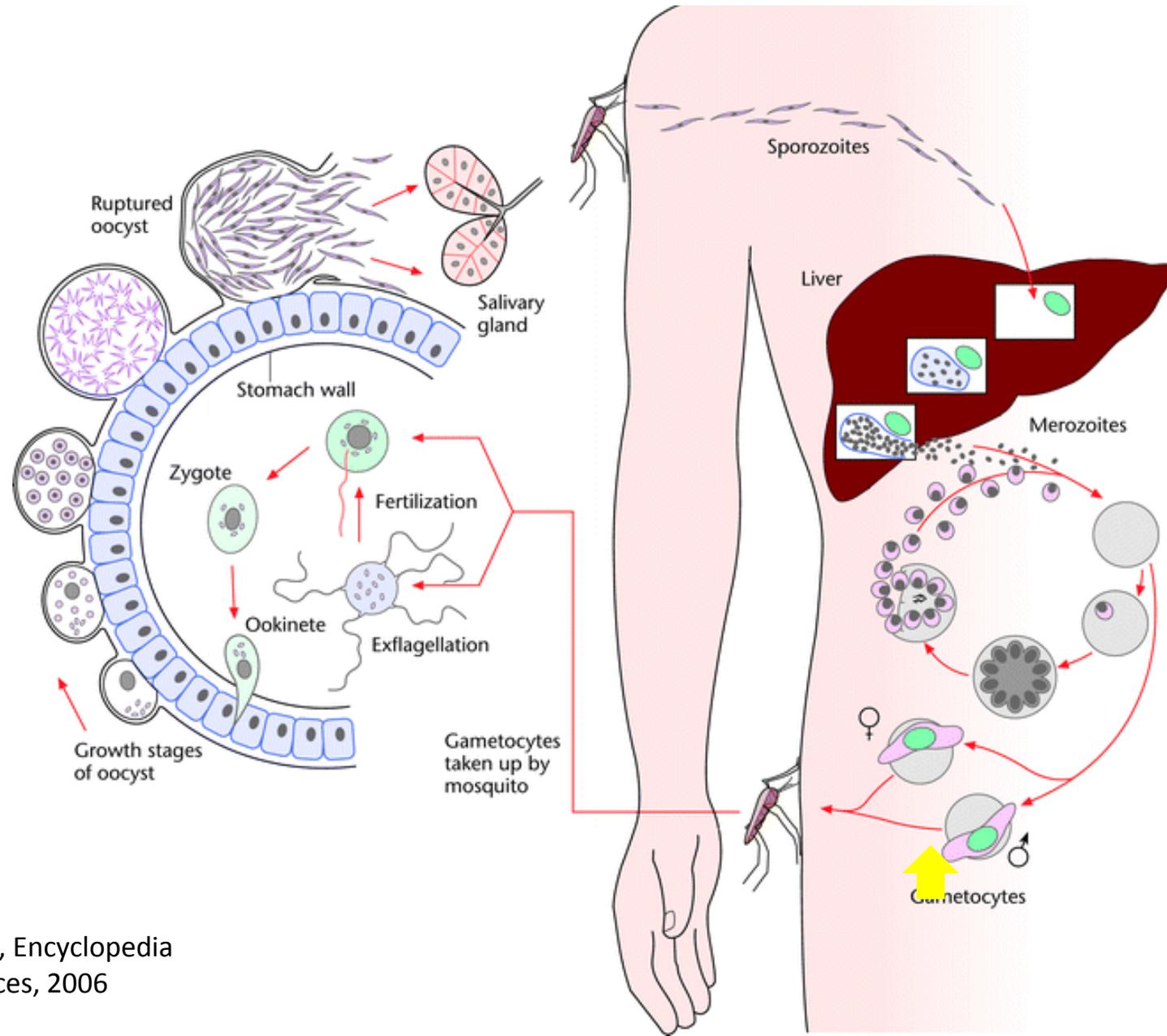


Life Cycle of Plasmodium in Mammals and Mosquito

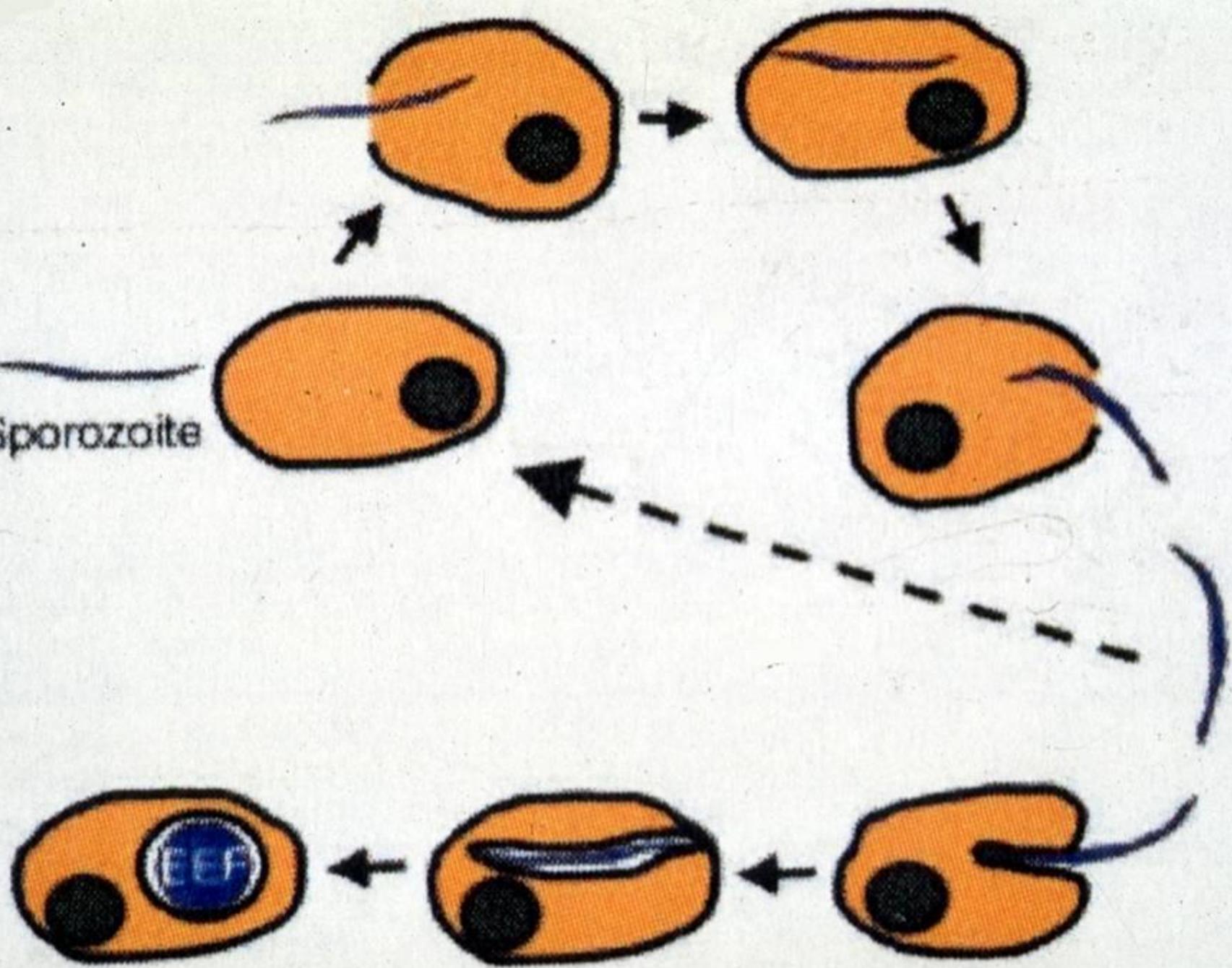


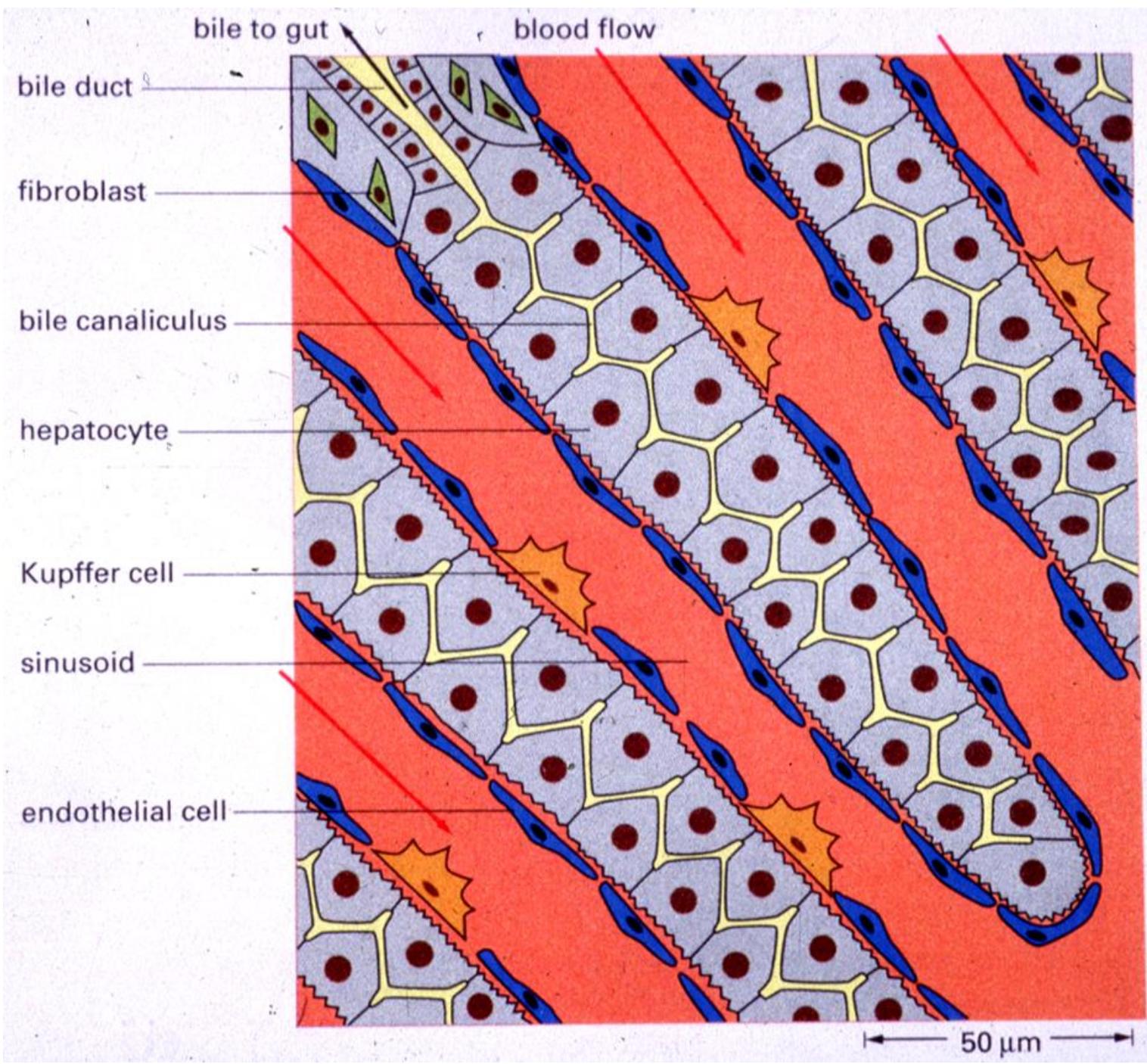


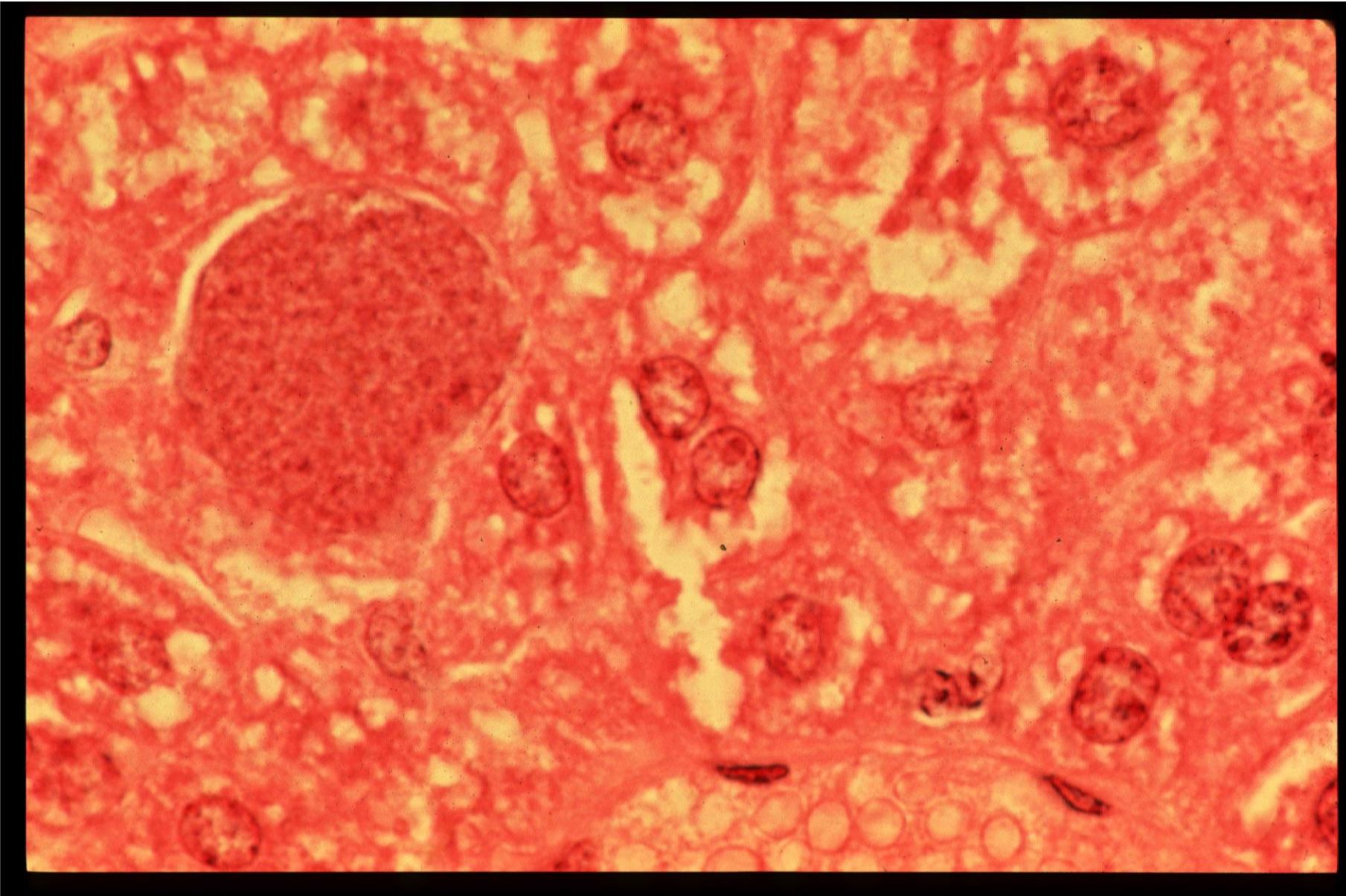
Properties of sporozoites

- **Gliding motility: no cilia or flagella, but move between cells (or on glass) faster than leukocytes**
- **Cell crossing: traverse cells rapidly. But only complete the development inside liver hepatocytes**
- **Bear an actin/myosin motor beneath the plasma membrane**

Sporozoite







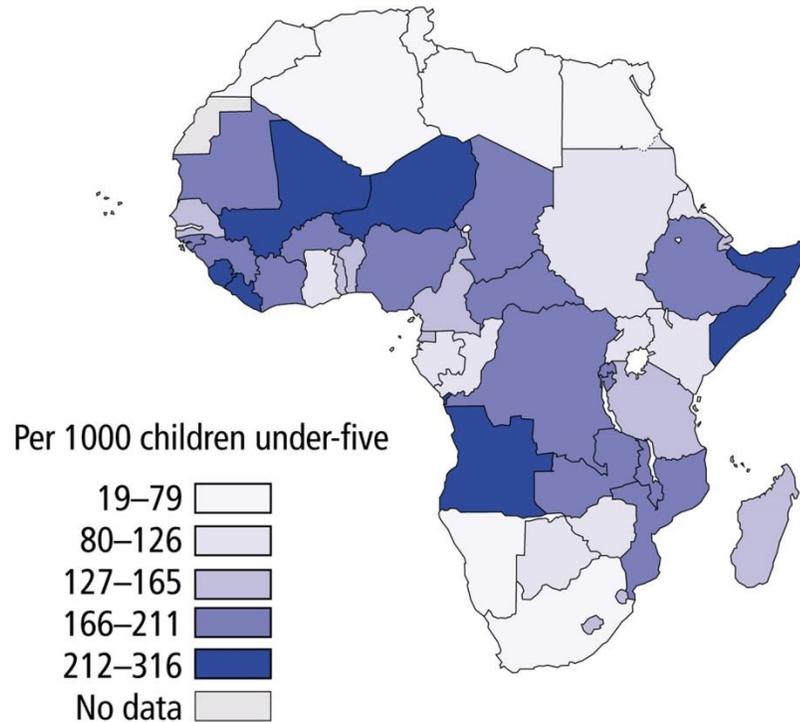
P. falciparum infections (2010)

216 million cases

655,000 deaths

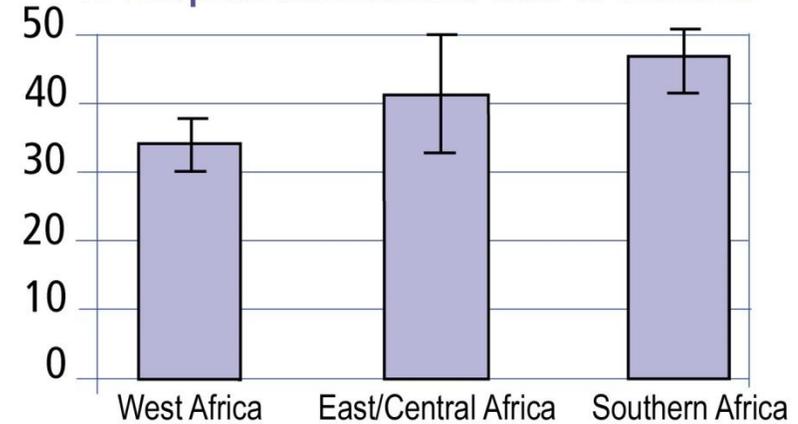
Plasmodium falciparum Malaria in Africa

under 5 years-old all cause mortality

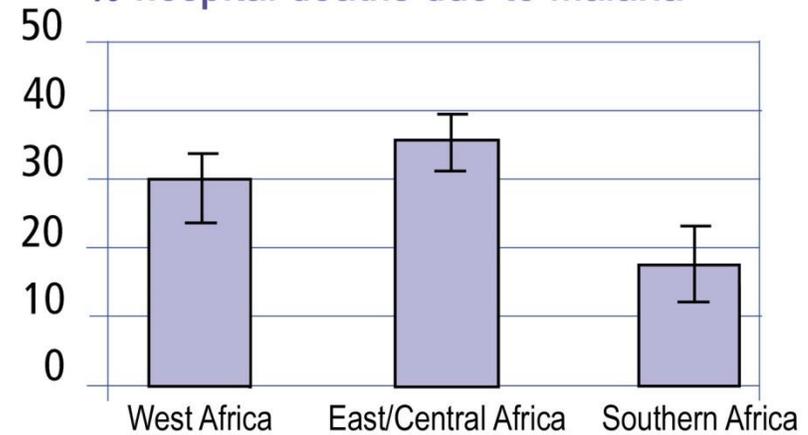


UNICEF (2003) State of the World's Children

% hospital admissions due to malaria



% hospital deaths due to malaria



WHO (2003) The Africa Malaria Report

Plasmodium vivax

Usually non-lethal, but quite virulent; prevalent in the Amazon region.

Dormant stages (hypnozoites). Frequent relapses.

Difficult treatment: Primaquine is the drug of choice.

•

Malaria prophylaxis

Impregnated bednets, elimination of mosquito breeding places,
DDT spraying of houses

Vaccine?

Old dogma

Protective immunity against malaria is very difficult or impossible to achieve since in endemic areas individuals can have multiple malaria attacks throughout their lives.

NATURE, VOL. 216, OCTOBER 14, 1967

Nussenzweig, Ruth et al.

**Protective Immunity produced by the
Injection of X-irradiated Sporozoites
of *Plasmodium berghei***



ARMY DOCTOR WATCHES MALARIA-CARRYING MOSQUITOES BITE STOMACH OF RICHARD KRICKENBÖCKER, SERVING 33 TO 34 YEARS AT ILLINOIS STATE PRISON

PRISON MALARIA

Convicts expose themselves to disease so doctors can study it

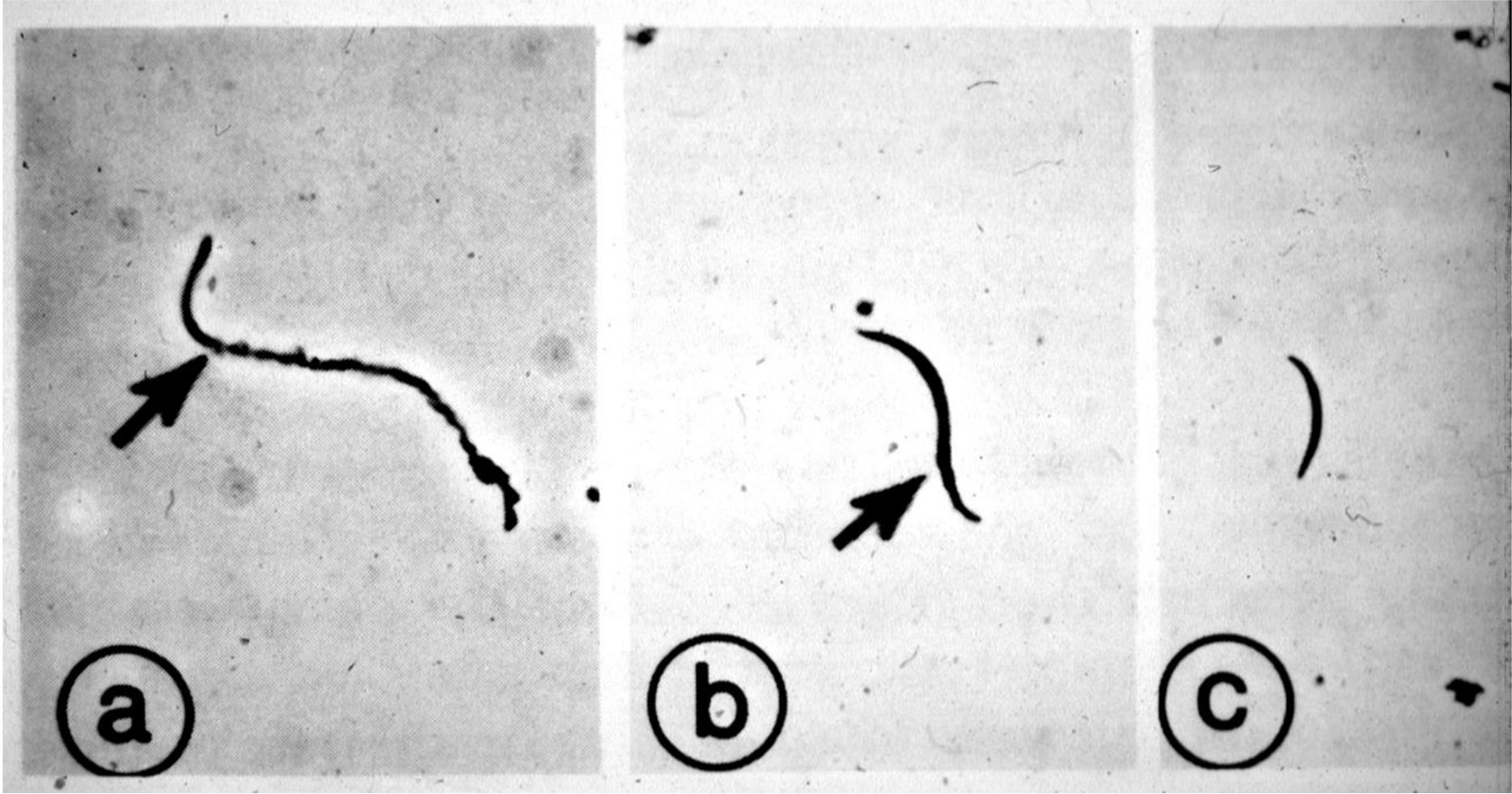
In three U. S. penitentiaries men who have been imprisoned as enemies of society are now helping advance fight another enemy of society. At the U. S. Penitentiary in Atlanta, the Illinois State Penitentiary and New Jersey State Reformatory some 500 convicts have volunteered to be infected with malaria so medical men can study the disease. The experiments, which are directed by the Office of Scientific Research and Development, have found prison life ideal for controlled laboratory work with humans.

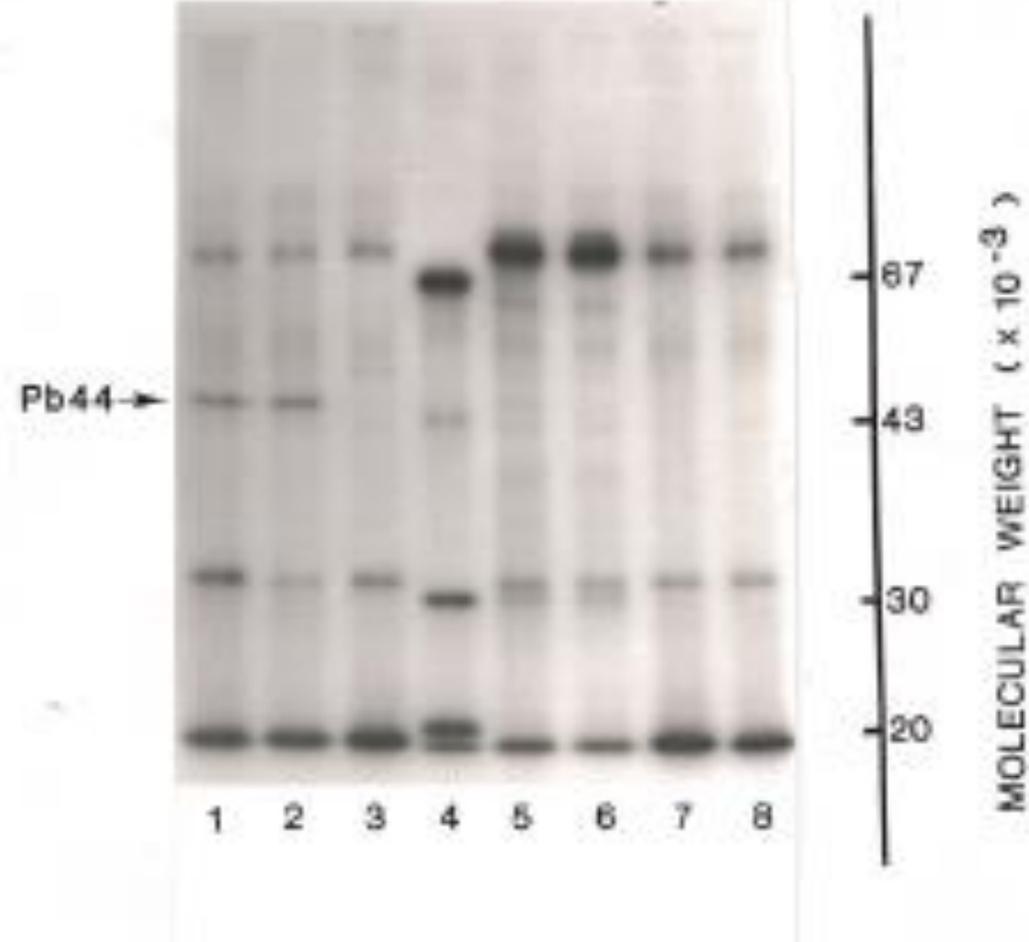
They subjects all eat the same food, sleep the same hours and exercise the same way. The prisoners are not punished or punished for volunteering to infection.

Prison malaria experiments emphasize the fact that malaria is still a very serious medical problem. In the U. S. there are 1,000,000 cases a year. The existing drugs usually combine and provide controlled malaria but cannot keep it from recurring long after the original infection. The goal of malaria research is to find a new drug which will cure the disease permanently.

CHARACTERISTICS OF SPOROZOITE-INDUCED IMMUNITY

- ① Stage - Specific.
- ② Species, But Not Strain Specific.
- ③ Protection Obtained by Intravenous Route or by Bite of Infected Mosquitoes.
- ④ Presence in the Serum of Protected Animals of Antibodies to the Surface of Sporozoites.

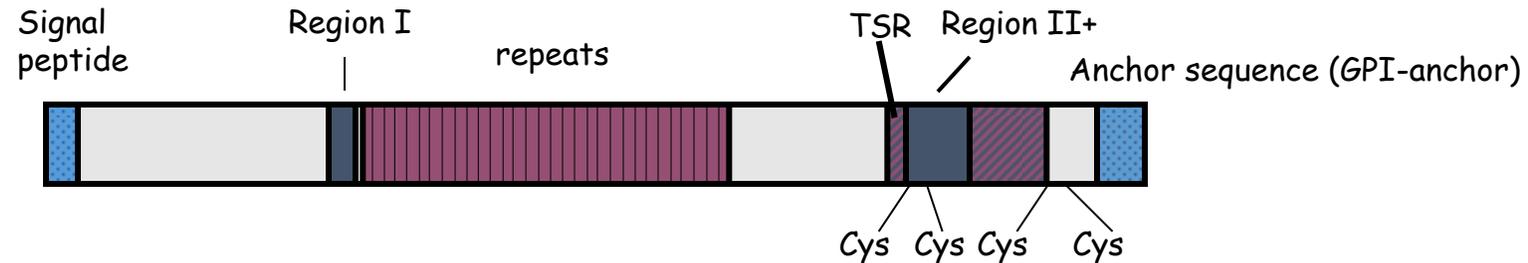




Circumsporozoite (CS) protein

- ❖ Major surface protein in sporozoite. Secreted in the medium.
- ❖ Encoded by a single copy gene.
- ❖ Expressed in mosquito sporozoites.
- ❖ Are 10-20% of total proteins made by salivary gland sporozoites.

Circumsporozoite (CS) protein

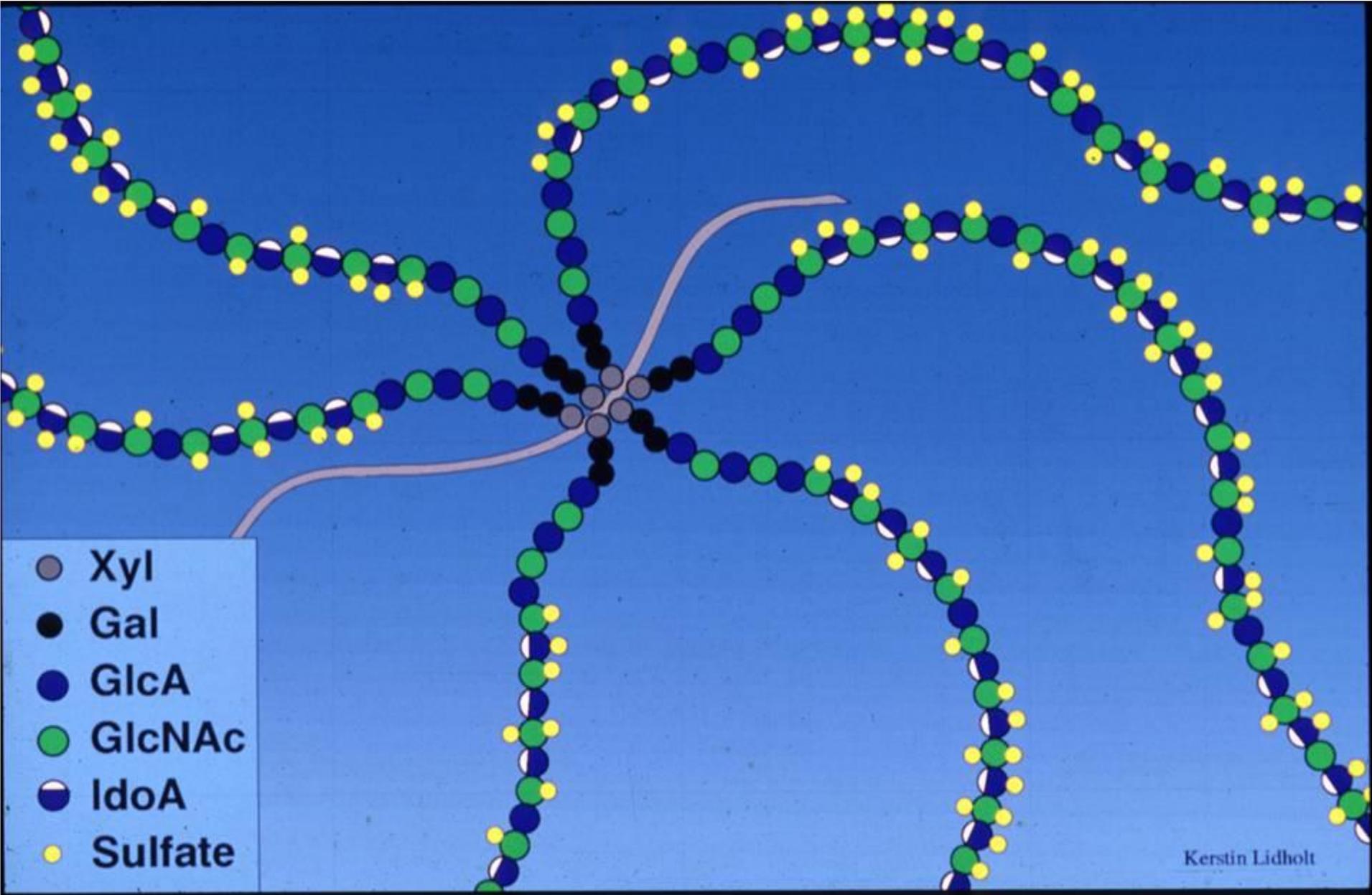


- ❖ N-terminal signal sequence.
- ❖ Central immuno-dominant repeats.
- ❖ Region I: KLKQP motif, highly conserved in CS from almost all species of *Plasmodium*.
- ❖ Region II+: contains the motif to bind to heparan sulfate proteoglycans (HSPGs) in the host's liver.
- ❖ C-terminal GPI-anchor signal sequence.

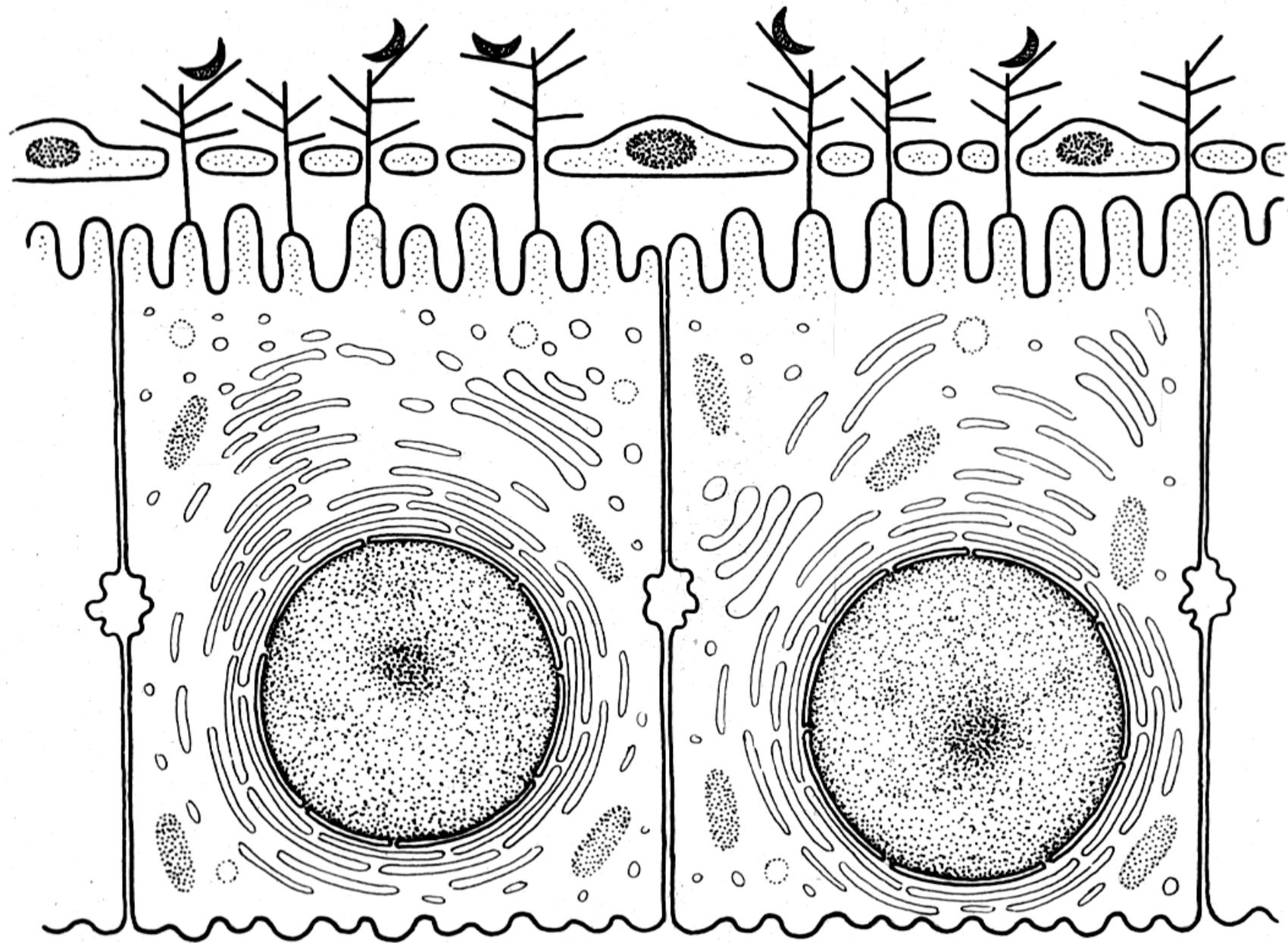
Region II+ of CS protein

Region II+ is homologous to the type I repeat of thrombospondin (TSR), a module found in a superfamily of adhesive proteins. The basic function of TSR is to mediate binding to sulfated glycoconjugates.

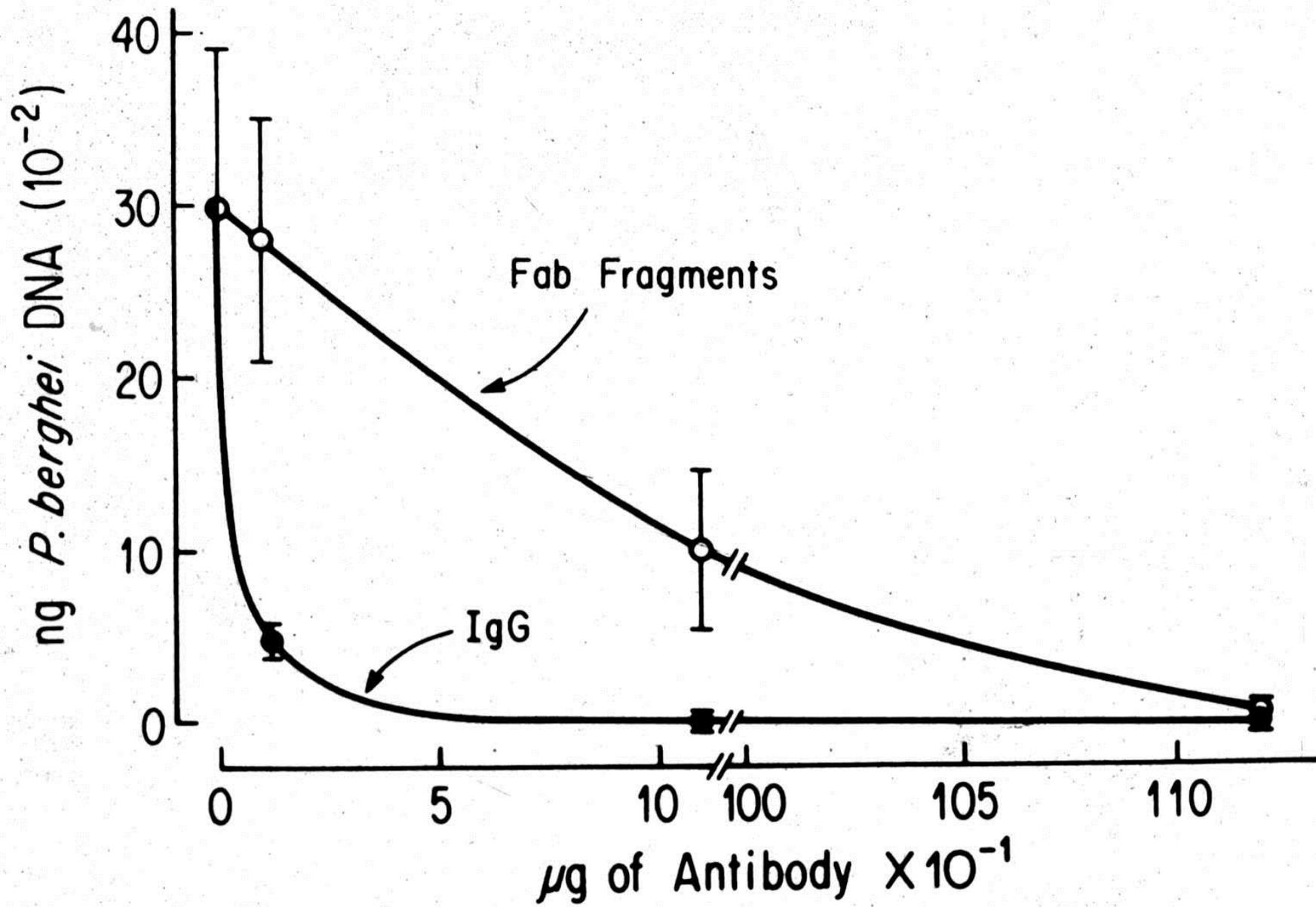
P. falciparum	EWSPCSVT CGNGIQVRIK
P. vivax	EWTPCSVT CGVGVVRRR
P. malariae	EWSPCSVT CGSGIRARRK
P. knowlesi	EWTPCSVT CGNGVRIIRK
P. cynomolgi	EWSPCSVT CGKRVRMRRK
P. brasilianum	EWSPCSVT CGSGIRARRK
P. berghei	EWSQCNVT CGSGIRVRKR
P. yoelii	EWSQCSVT CGSGVVRKR
TRAP/SSP2	EWSPCSVT CGKGT RSRKR
Etp100	EWTECSAT CGGGT KHREER
Thrombospondin	PWSSCSVT CGDGVITRIIR
Properdin	PWGP CSVT C SKGTQORQR
Complement Protein 6	QWTS CSKT CNSGTQS RHR
Unc-5	DWSACSSSC --HRYRTRA
F-spondin	EWSDCSVT CGKGM --RTR



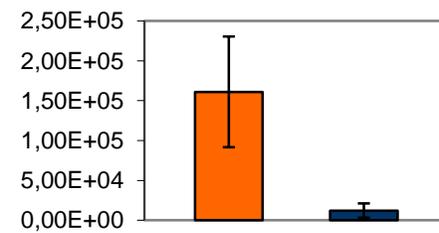
Kerstin Lidholt



CS protein has a central role in sporozoite
invasion of hepatocytes



Infectivity of sporozoites is strongly inhibited in CS-transgenic mice



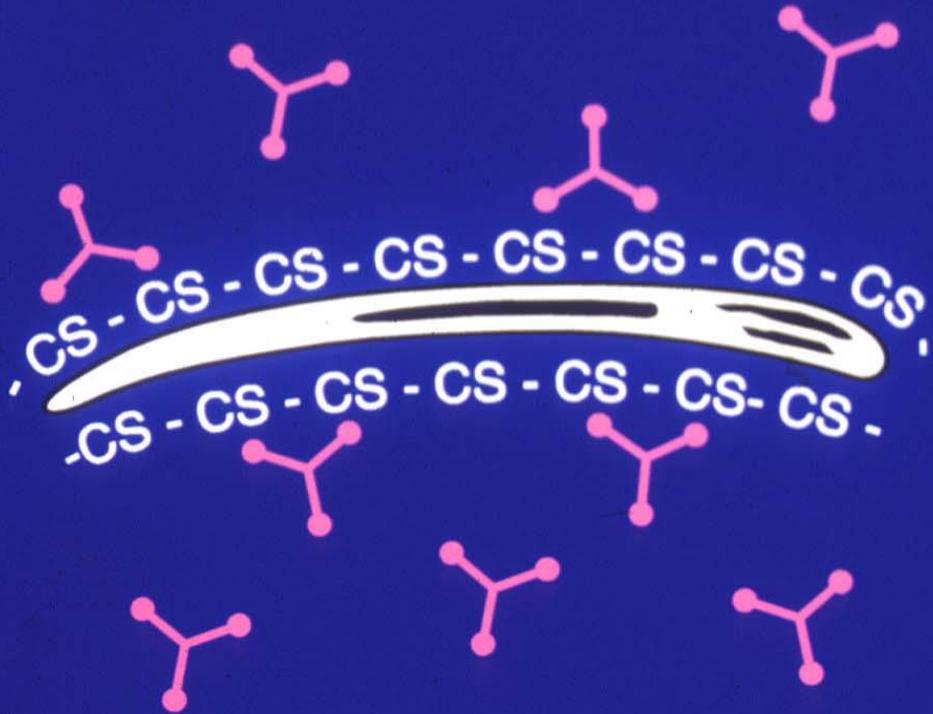
Inhibition of Development of Exoerythrocytic Forms of Malaria Parasites by γ -Interferon

ARTURO FERREIRA, LOUIS SCHOFIELD, VINCENZO ENEA,
HUUB SCHELLEKENS, PETER VAN DER MEIDE, WILLIAM E. COLLINS,
RUTH S. NUSSENZWEIG, AND VICTOR NUSSENZWEIG

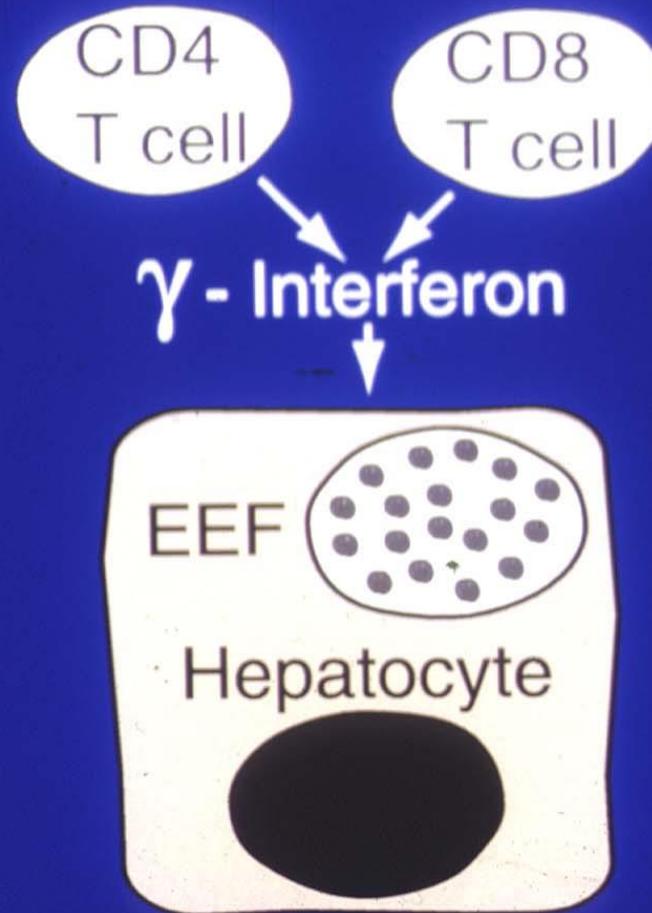
Science 232, 881-4, 1986

Protection mediated by irradiated sporozoites

step 1



step 2



However....

Millions of lymphocytes are required to find and destroy the rare liver stages among 10^9 hepatocytes.

(NANP)₃-tetanus toxoid vaccine

Hoffman-La-Roche and NYU

3/14 volunteers had ELISA titers above 1/500.

**Upon challenge: in one volunteer protection was sterile,
and in the other two it was partial.**

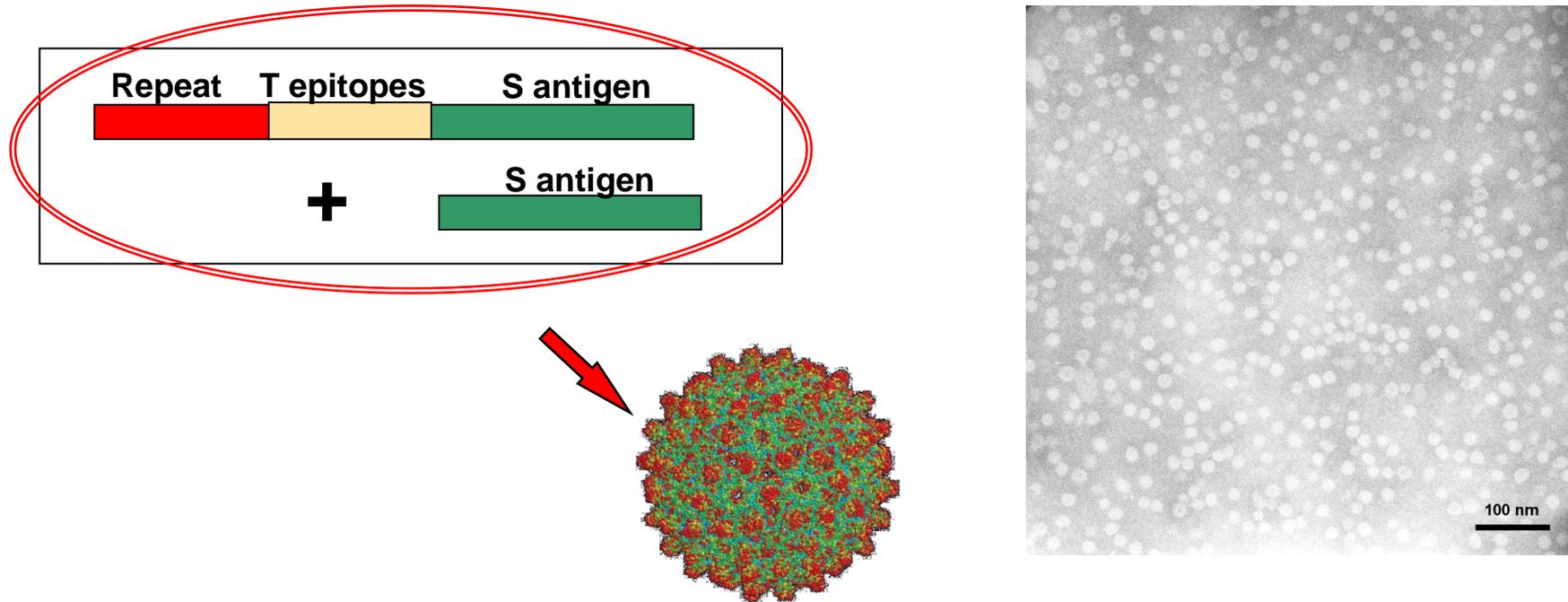
Protection was 100% antibody-mediated.

RTS,S vaccine

USA army (Ripley Ballou) and GSK (Joe Cohen)

Adjuvant: liposome-based, contains
immunostimulants

The RTS,S antigen

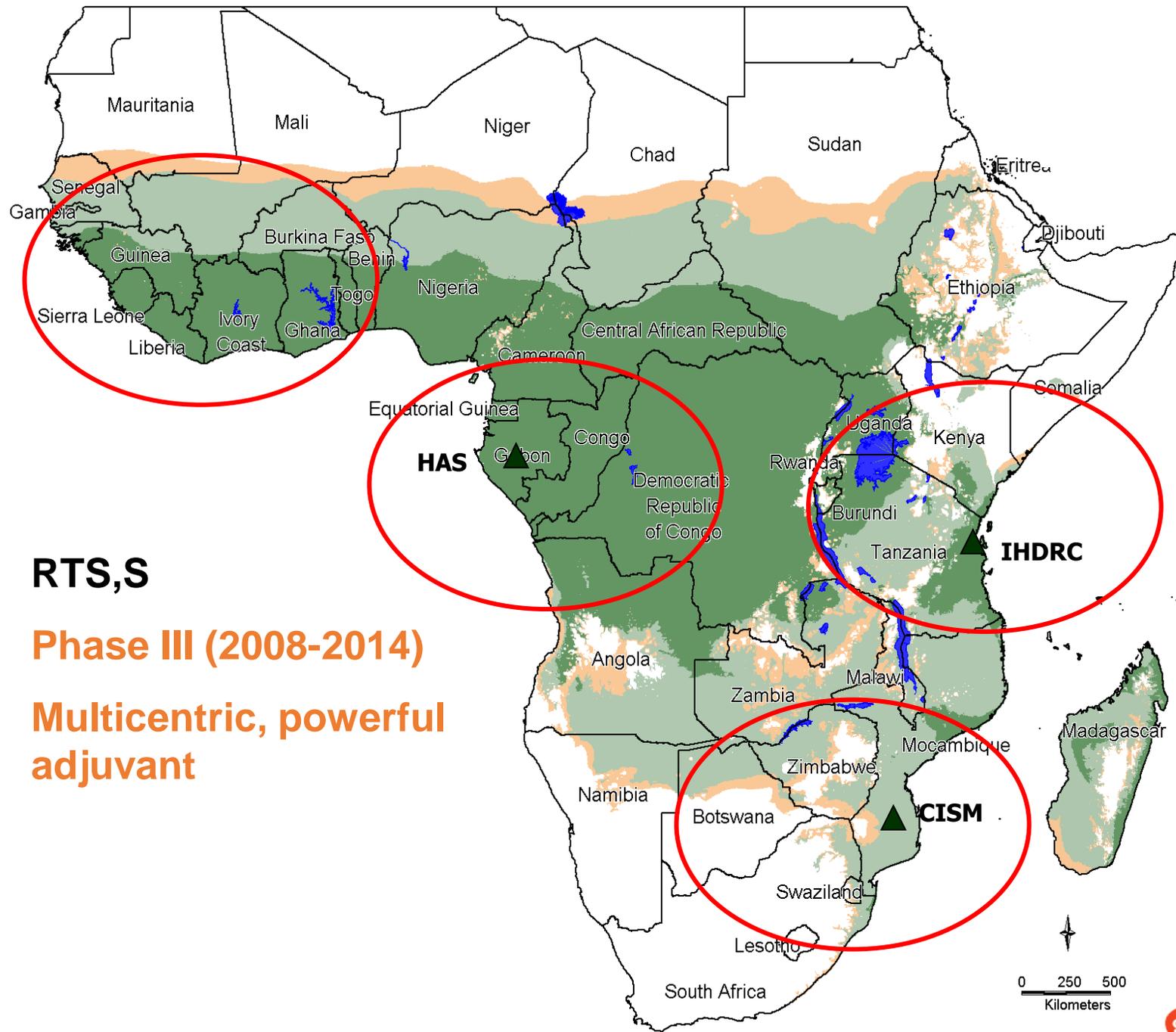


- Expressed in recombinant yeast (*S. cerevisiae*)
- RTS,S particles assemble spontaneously

Vaccine efficacy in children

**Against clinical disease, severe malaria:
30-50%**

**Significant correlation between
antibody levels and protection.**



RTS,S

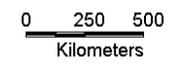
Phase III (2008-2014)

Multicentric, powerful adjuvant

HAS

IHDC

CISM



Problems for RTS,S

Sporozoites can enter hepatocytes in a few minutes and are then protected from antibodies. Very high levels of serum antibodies are required to neutralize all injected sporozoites.

T cells secreting interferon gamma destroy liver stages. But very large numbers of T cells are needed to encounter the few parasites among millions of hepatocytes.

PROTECTION AFTER CHALLENGE WITH 5-14 INFECTED MOSQUITOES

Stephen Hoffman (review)

<u># IMMUNIZING EXPOSURES</u>	<u># VOL PROTECTED/ # VOL CHALLENGED</u>	<u># PROTECTED CHALLENGES/ # CHALLENGES</u>
> <u>1000 Infected Mosquitoes</u>		<u>Total: 33/35</u>
1 st challenge	13/14 (93%)	13/14 (93%)
Re-challenge <10 wk	6/6 (100%)	15/15 (100%)
Re-challenge 23-42 wk	5/6 (83%)	5/6 (83%)
< <u>1000 Infected Mosquitoes</u>		<u>Total: 5/15</u>
1 st challenge	4/10 (40%)	4/10 (40%)
Re-challenge <10 wk	1/1	1/1
Re-challenge >10 wk	0/3	0/4

Attenuated sporozoite vaccines (SANARIA).

**Sporozoites have to be free of contaminants.
Sanaria OK**

Irradiation has to be well controlled. Sanaria OK

**Sporozoites have to be kept frozen until used
Sanaria OK.**

**6-10 boosters are needed to achieve complete
protection. Sanaria OK**

Protection decays between 6-12 months.

**Protection requires intravenous
immunization**

Conclusions

The circumsporozoite protein is a protective antigen.

RTS,S provides partial protection, and it does not last long enough. Protection is mainly or exclusively antibody-mediated.

Attenuated sporozoite vaccines are unlikely to be used in endemic areas

Perspectives

Combine RTS,S with new protective antigens from liver stages or blood stages.

Use a new and more powerful adjuvant.

Boost RTS,S with a viral CS vector.

Identification of sporozoite species in
mosquitoes from endemic areas

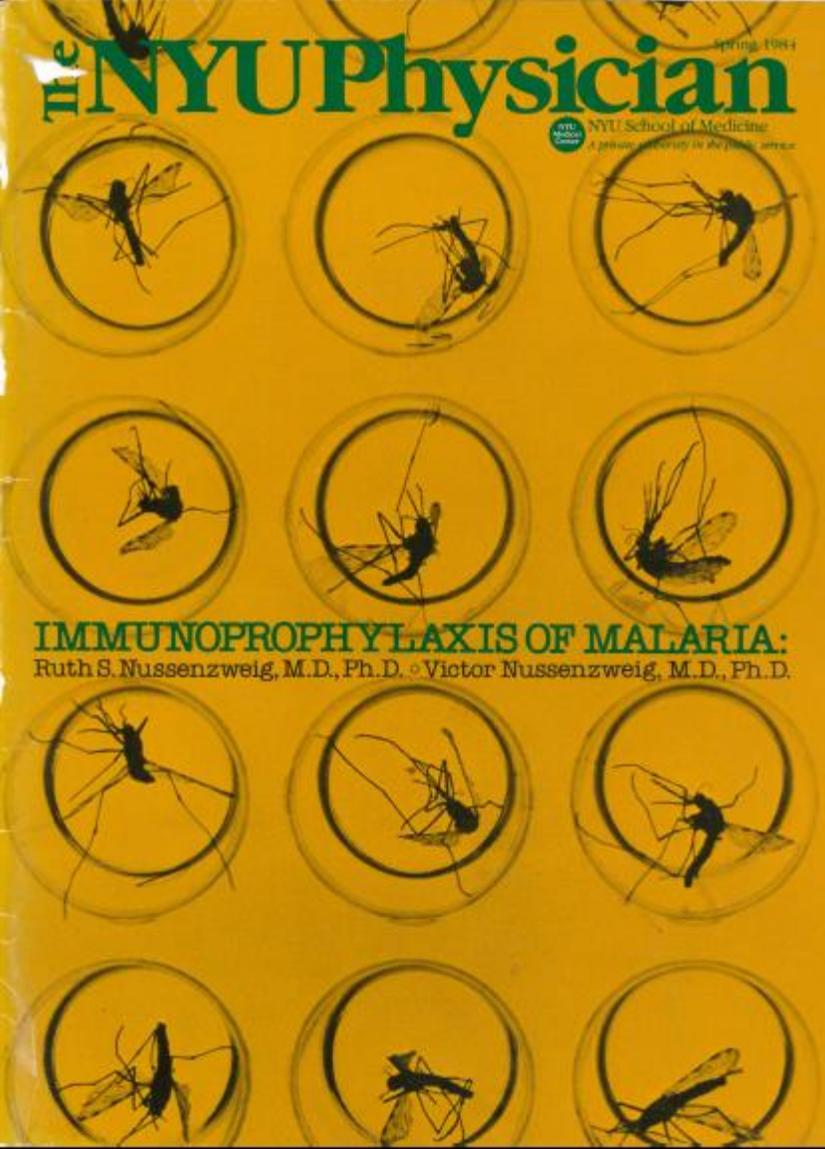
|

**In the past required laborious dissection of
salivary glands and microscopic examination.**

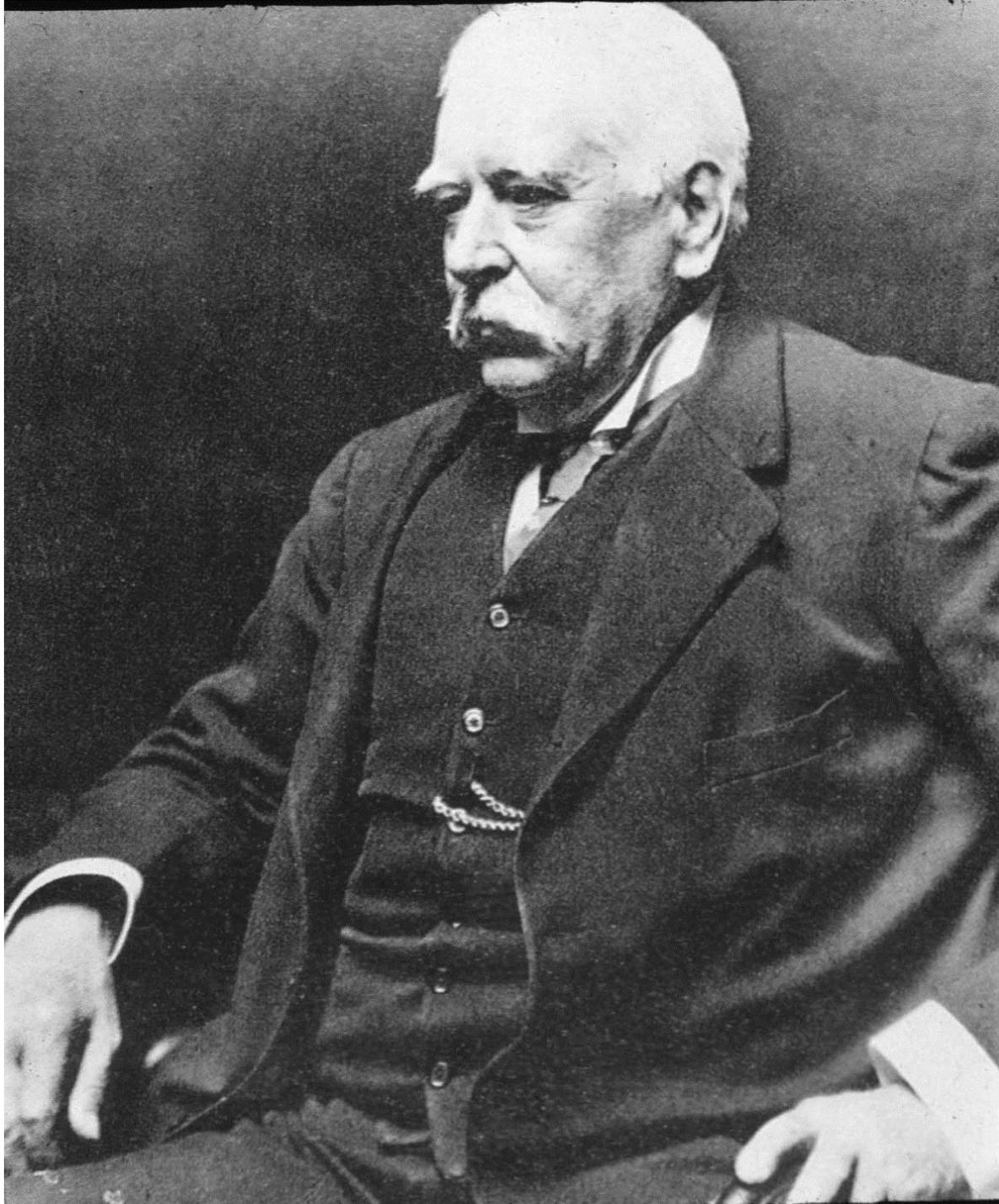
Spring 1984
the NYU Physician

NYU School of Medicine
A Division of the University of the Health Sciences

IMMUNOPROPHYLAXIS OF MALARIA:
Ruth S. Nussenzweig, M.D., Ph.D. • Victor Nussenzweig, M.D., Ph.D.

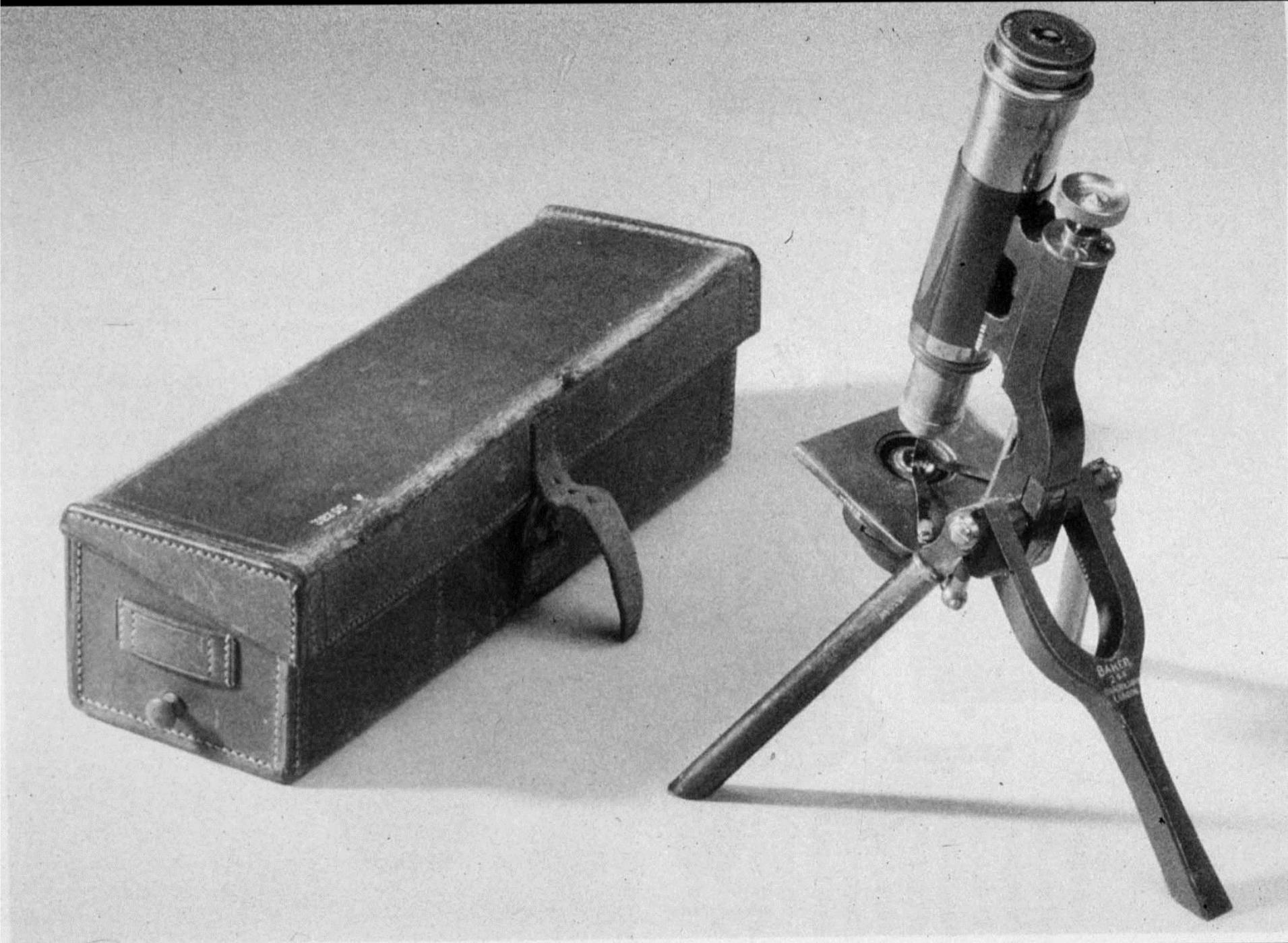






Patrick Manson's theory in a letter to Ross, July 17th 1895.

...the debris of dead mosquitoes in which the germ had become encysted penetrate the human mucous membrane and get into circulation. ...evaporate some water in which malariated mosquitoes had died, send to England; I am willing to drink it.



Ronald Ross to Patrick Manson describing the struggle between a male gametocyte and phagocytes
(July 30th 1895).

...I watched it for three solid hours exactly.

..he (the gametocyte) brought up against a phagocyte...he kept poking him in the ribs. I was astonished; and so, apparently, was the phagocyte... After 50 minutes the beast seemed to be getting tired, ...when a third phagocyte came at him with the mouth open right and straight across the field...but the flagellum attacked, holding on and shaking. In one minute the third phagocyte turned sharp round and ran howling!!!

I assure you. I won't swear I heard him howling, but I *saw* him howling.... I shall dream of it.

I shall write a novel on it in the style of *The Three Musketeers*..

By the way, this *entre nous*, I would not give myself away too much to Laveran. He seems to have a disposition to collar as much as he can for himself. This of course is very confidential. Send him the thing complete but not in bits as he might use your bricks to build his own house. I don't want to be ungenerous but I want you to get the full credit for work which is yours and not his.

Patrick Manson to Ross, April 9, 1899