Metodologie di Sintesi e Sviluppo Farmaceutico

Synthesis and Development Pharmaceutical Methodologies

Laurea Magistrale in Chimica a.a. 2018/2019

Medicinal chemistry historical evolution

Targeting Malaria by natural sources and by rational design

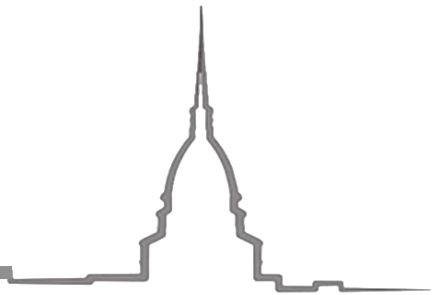
Marco L. Lolli University of Torino (UniTO)

Definition of Medicinal Chemistry

"Medicinal chemistry concerns the **discovery**, the **development**, the **identification** and the interpretation of the mode of action of biologically active compounds at the molecular level.

Emphasis is put on drugs, but the interests of the medicinal chemist are not restricted to drugs but include **bioactive compounds** in general.

Medicinal chemistry is also concerned with the study, identification, and synthesis of the **metabolic products** of these drugs and related compounds."



The story of the Medicinal Chemistry:
the alchemic times

The Alchemist (N. C. Wyeth – 1937)

Someone that pay the bill

The freedom of judgement

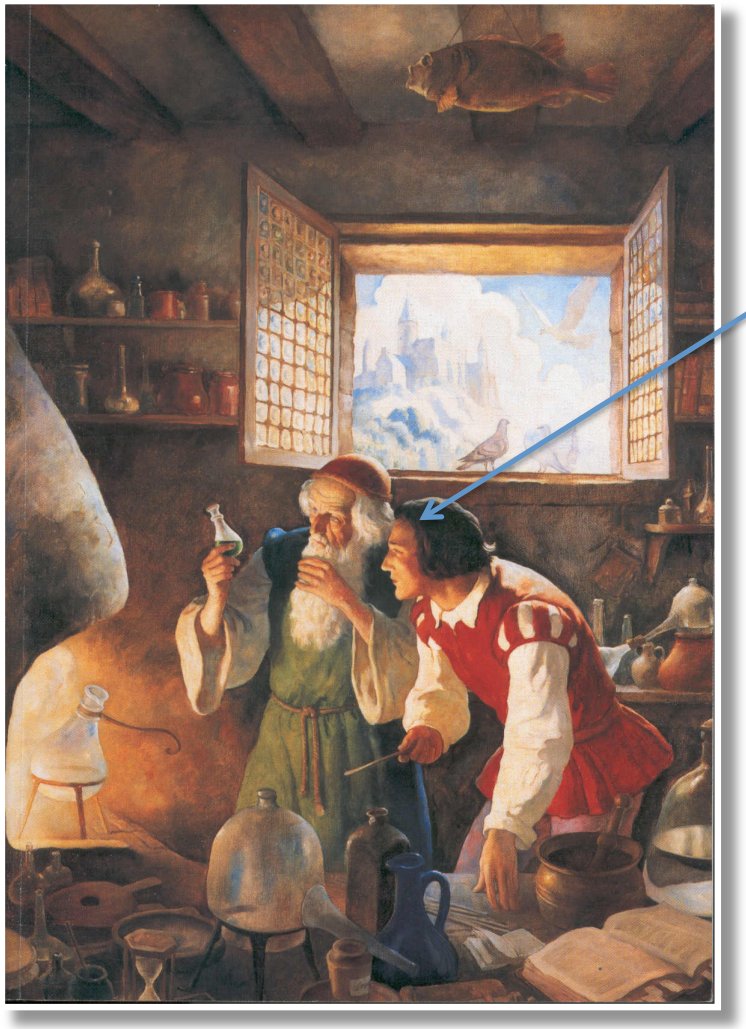
A knowledge transfer

Bibliography is important

Marco L. Lolli

University of Torino (UniTO)

The story of the Medicinal Chemistry:
the alchemic times



In <u>absence</u> of science rules, the solubility phenomena was absolutely **amazing**!!!

…..A mineral white powder, formed by evaporating seawater, resist to hot flames but disappear (transform) if water is added…...

- *Alkahest*

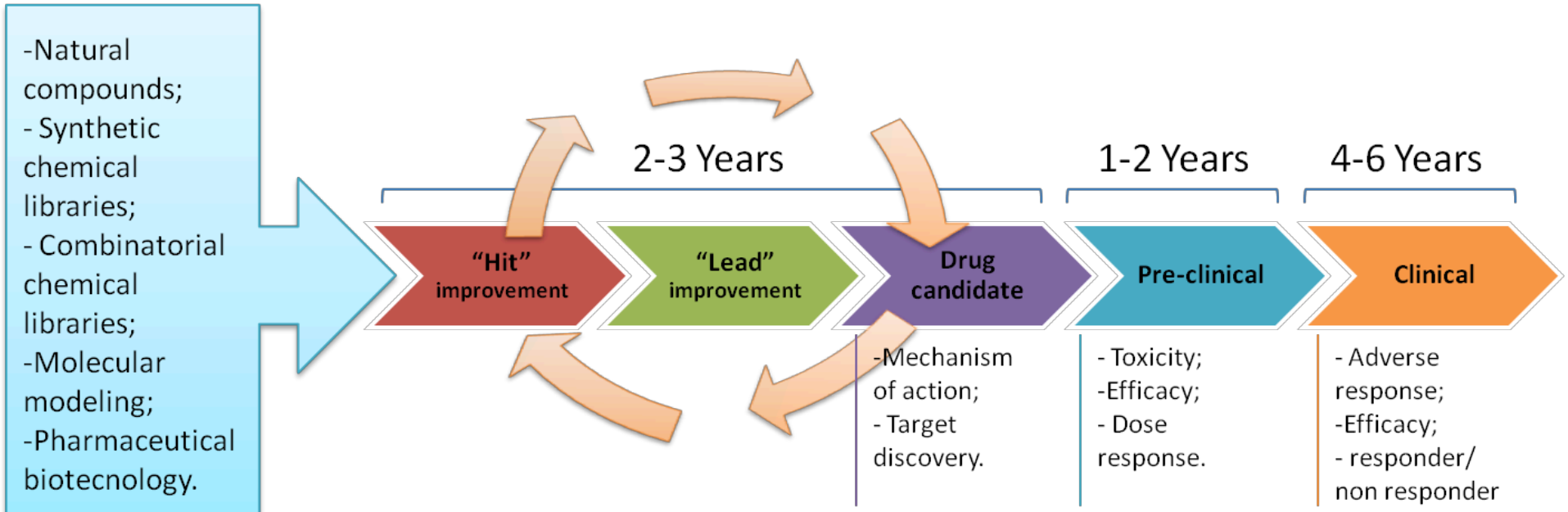
- *Similia similibus solvuntur*

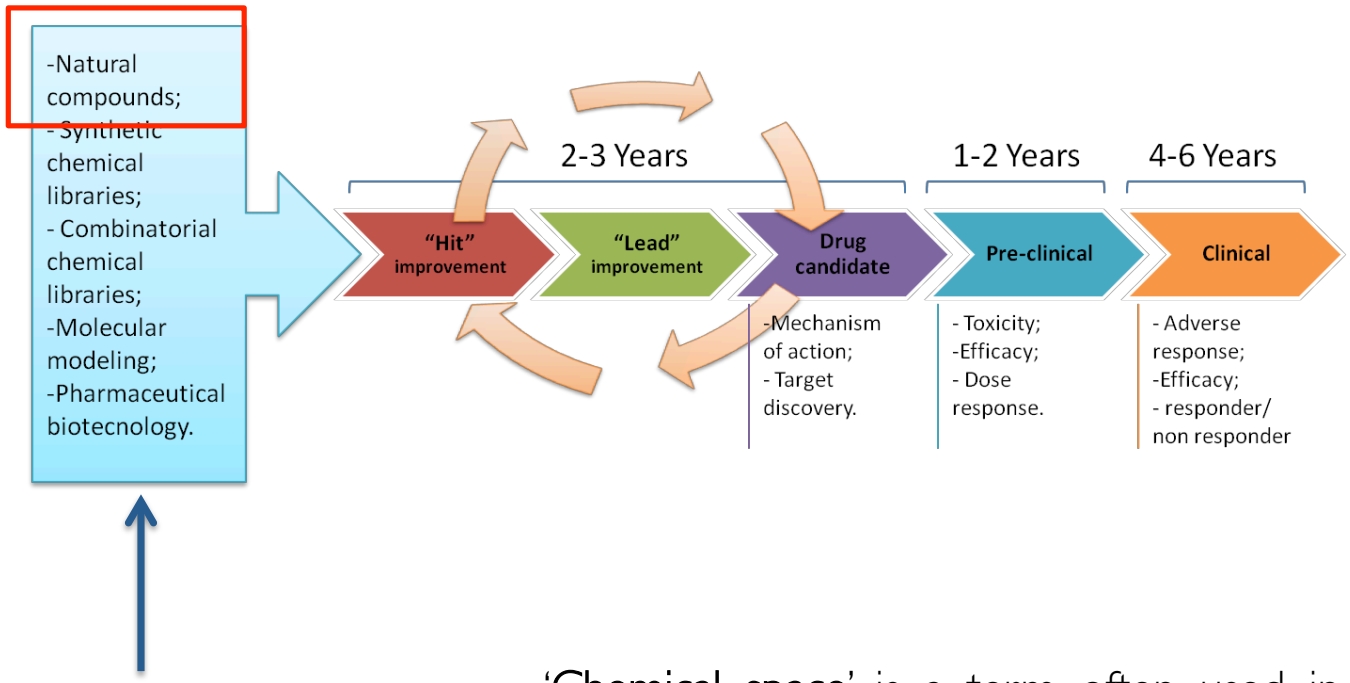
The Alchemist (N. C. Wyeth – 1937)

The story of the Medicinal Chemistry:
first documents

(a) Cuneiform clay tablets unearthed from the library of King Ashurbanipal at Nineveh describe Mesopotamian medical practices. It is estimated that they originated between 1900 and 1700 BC.
(b) A section of the Ebers Papyrus, a compilation of Egyptian medical knowledge composed around 1550 BC containing over 800 prescriptions for various conditions
(c) A page from the Pen-tsao Kang-mu, a compilation of traditional Chinese medicines written by Li Shih-chen.

The drug design process

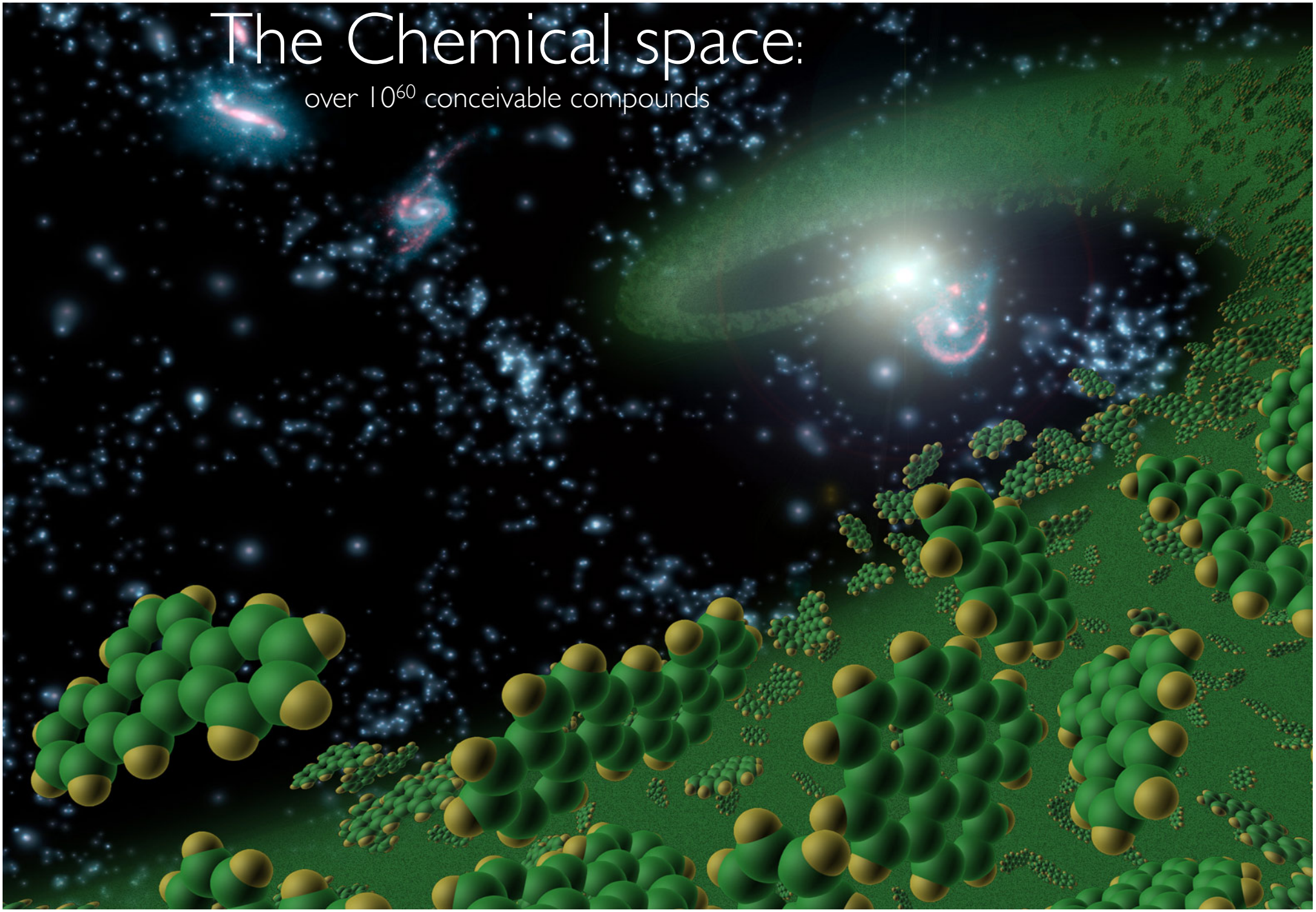




Chemical space

'Chemical space' is a term often used in place of '**multi-dimensional descriptor space**': it is a region defined by a particular choice of descriptors and the limits placed on them.

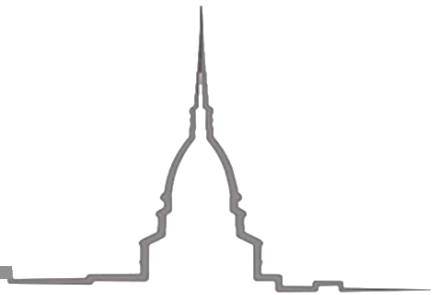
Refining the chemical toolbox for drug discovery in the 21st Century
Marco L. Lolli, Sarah Narramore, Colin W.G. Fishwick and Klaus Pors
Drug Discovery of Today **2015**, 20(8), 1018


The Chemical space:
over 10^{60} conceivable compounds

The story of the Medicinal Chemistry:
the plants roles

"*Dio ha creato tutte le malattie, ma ha anche creato un agente o un farmaco per ogni malattia. In natura si può trovare ogni cosa, perché la natura è la farmacia universale*"
Paracelso – 1500

Therapeutic uses of erbs extracts



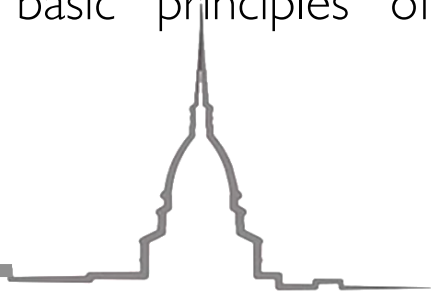
The story of the Medicinal Chemistry:
the plants roles

Advantages

- illimitate source of materials
- illimitate chemiodiversity

Weakness

- Difficulties to obtain pure chemicals with medicinal value extracts
- Medications defined using empirical observation of the presence or absence of symptoms in patients, rather than an understanding of the disease
- Absence of the vast majority of the fundamental knowledge required to understand even the basic principles of disease progression.

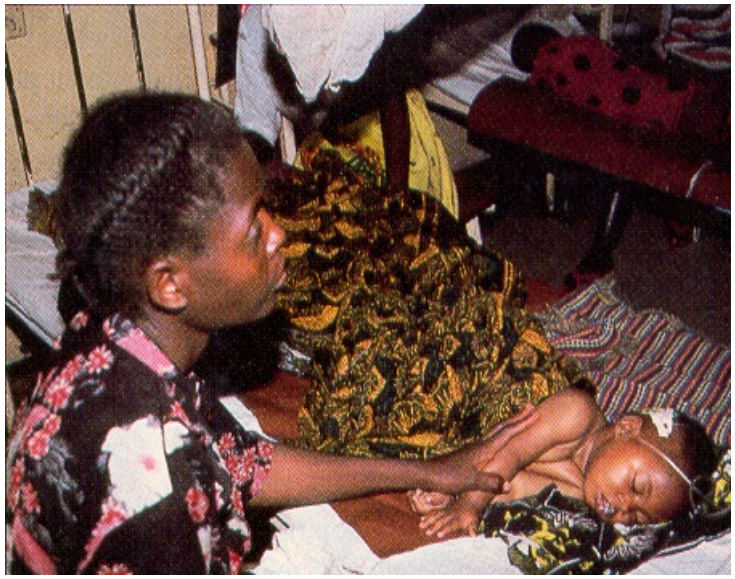


Malaria vs human race: a never ending story

The Malaria problem

Malaria is the most common life-threatening infection

- 1 million deaths/yr
- 300-500 million infections/yr
- ~90% of these deaths occur in sub-Saharan Africa
- most victims are children <5 yrs
- Pregnant women are also especially vulnerable.



The Malaria and the human race

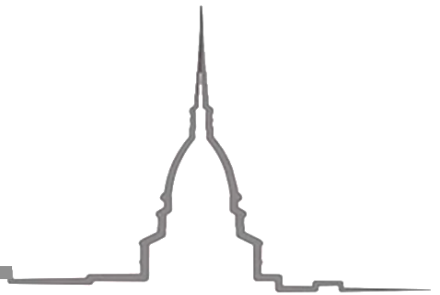
- Region between Tigris and Euphrates was malarious (2000 B.C.)
- Nei Ching Canon of Medicine (1700 B.C.)
- **Egyptian** mummies with enlarged spleens (1000 B.C.)
- ***Hippocrates*** (460-370 B.C.) described the malaria synthoms.
- **Alexander *the Great*** (356 - 323 B.C.)
- **American Civil War** (1861-1865): patients with mosquito nets (Washington D.C.)
- American soldier during the **Vietnam war**

Clinically mild Malaria

- An abrupt onset of an initial '***cold stage***' associated with dramatic rigors in which the patient visibly shakes;
- An ensuing '***hot stage***' during which the patient may have a temperature of well over 104°F (40°C), may be restless and excitable, and may vomit or convulse;
- Finally, the '***sweating stage***', during which the patient defervesce and may fall asleep;
- The symptom are **cyclic**, every three weeks (*terzana*) or every four week (*quartana*).

Severe Malaria

- Cerebral Malaria, with abnormal behavior, impairment of consciousness, seizures, coma, or other neurologic abnormalities;
- Severe anemia due to hemolysis;
- Hemoglobinuria;
- Pulmonary edema;
- Abnormalities in blood coagulation and thrombocytopenia;
- Cardiovascular collapse and shock;



Human Malaria is caused by one of four protozoan parasites:

Plasmodium falciparum

Plasmodium vivax

Plasmodium ovale

Plasmodium malariae

Malaria is **transmitted through the bite** of an infected female *Anopheles* mosquito

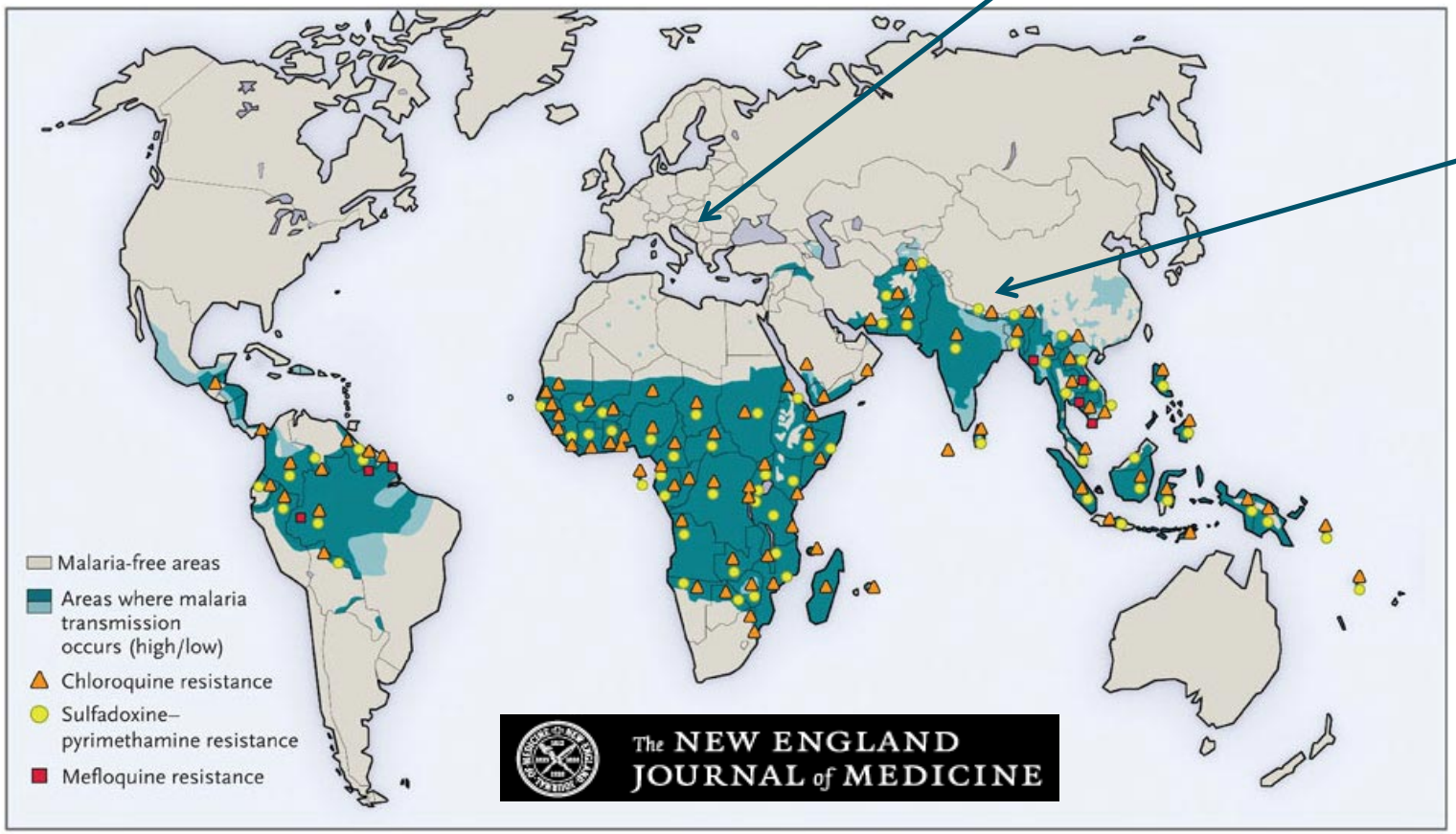
WHO Malaria definition:

"*Malaria is a disease of poverty that distort and lows a country's economic grow*"

The Malaria problem

Italy

India



WHO definition:

"*Malaria is a disease of poverty that distort and lows a country's economic grow*"

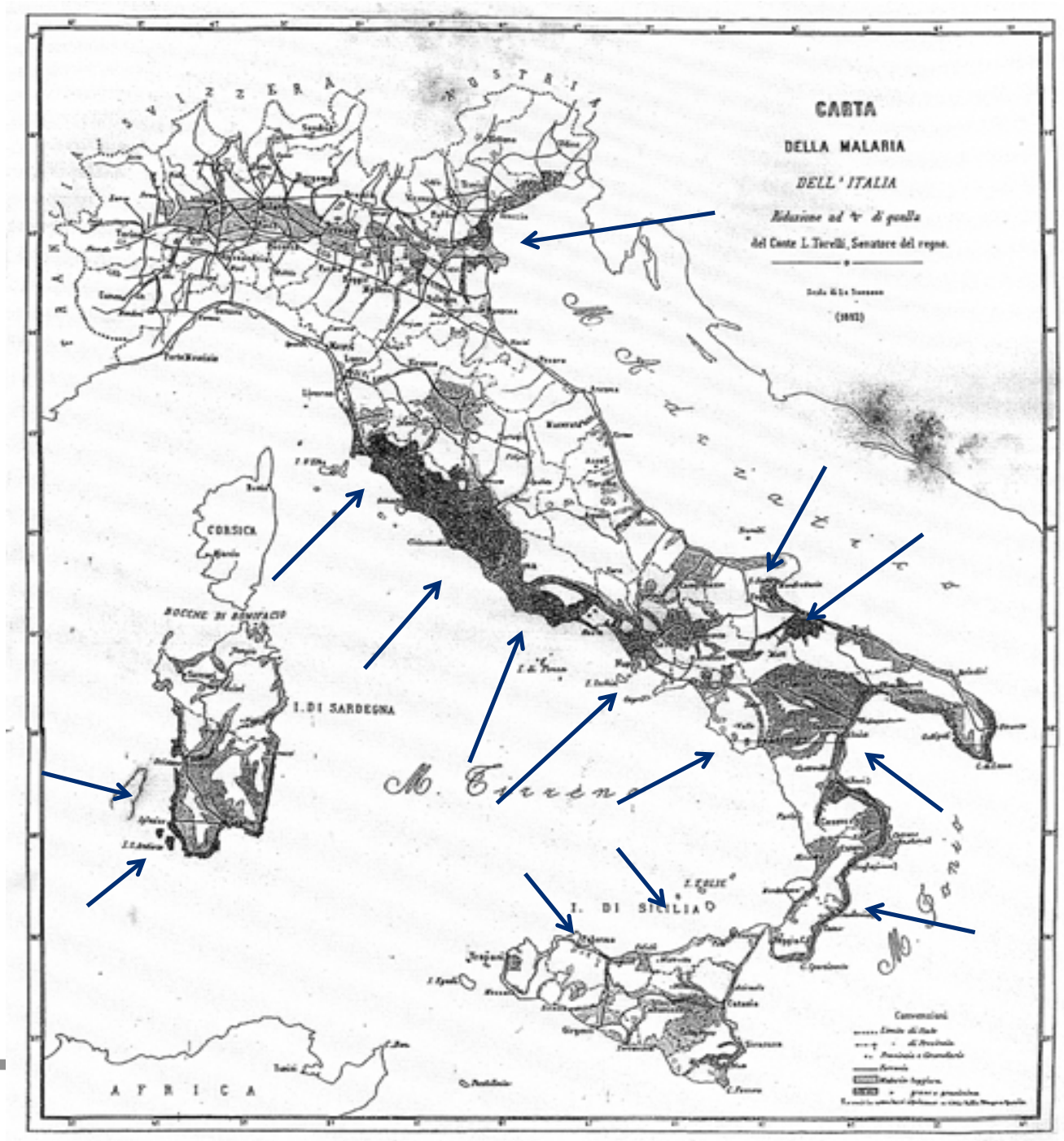
2008; 358: 2425-2428

Malaria and Italy

"*Paludismo*" (Swamp)

"*Mal-aria*" (bad air)

"*Tellurismo*"



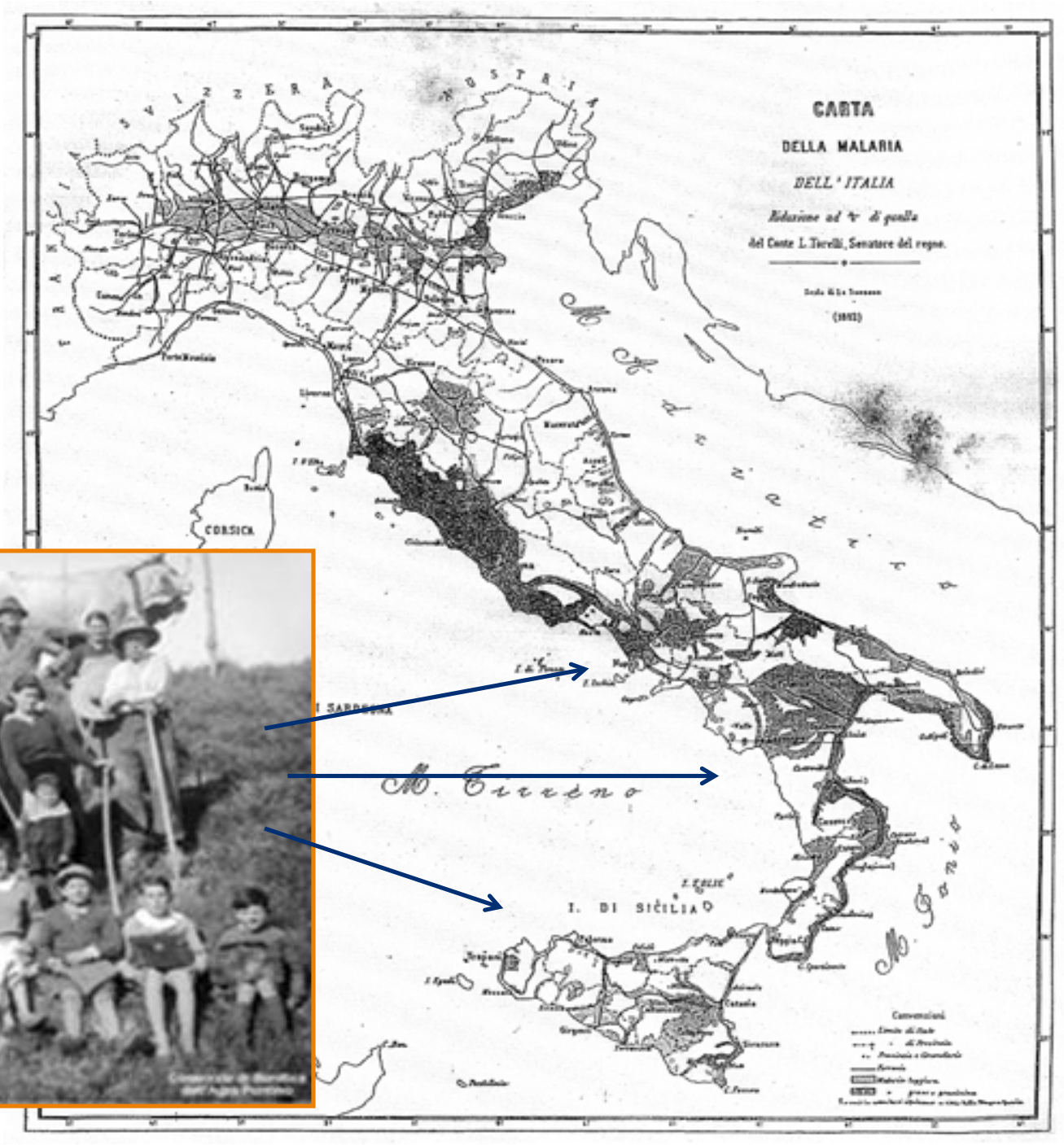
Malaria in Italy (1882)

Rice fields


CARTA
DELLA MALARIA
DELL' ITALIA
Riduzione ad 1/ di quella
del Conte L. Torelli, Senatore del regno.
(1883)

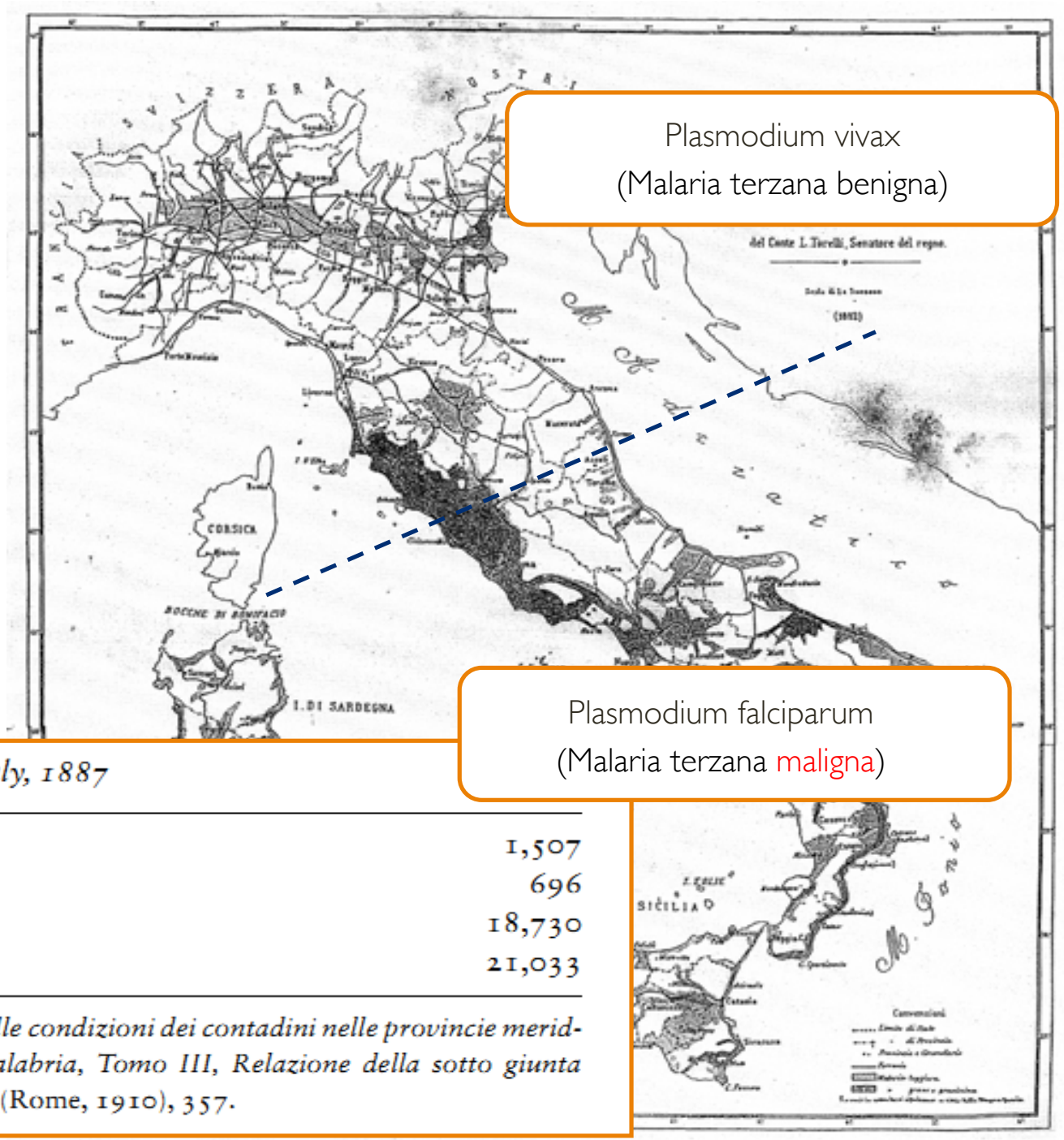
CIELO SULLA PALUDE
INES ORSINI - MAURO MATTEUCCI - GIOVANNI MARTELLA

Agricultural



The "*Agro pontino*" area around Rome

Two Italies with **different** Malaria



Plasmodium vivax
(Malaria terzana benigna)

Plasmodium falciparum
(Malaria terzana maligna)

Table 1.2 Mortality from Malaria in Italy, 1887

North	1,507
Center (minus Lazio)	696
South (plus Lazio)	18,730
Total (including Sicily and Sardinia)	21,033

Source: Data from *Inchiesta parlamentare sulle condizioni dei contadini nelle provincie meridionali e nella Sicilia*, vol. V, *Basilicata e Calabria, Tomo III, Relazione della sotto giunta parlamentare, Relatore: On. Francesco Nitti* (Rome, 1910), 357.

Escaping from Malaria since the beginning of XX century

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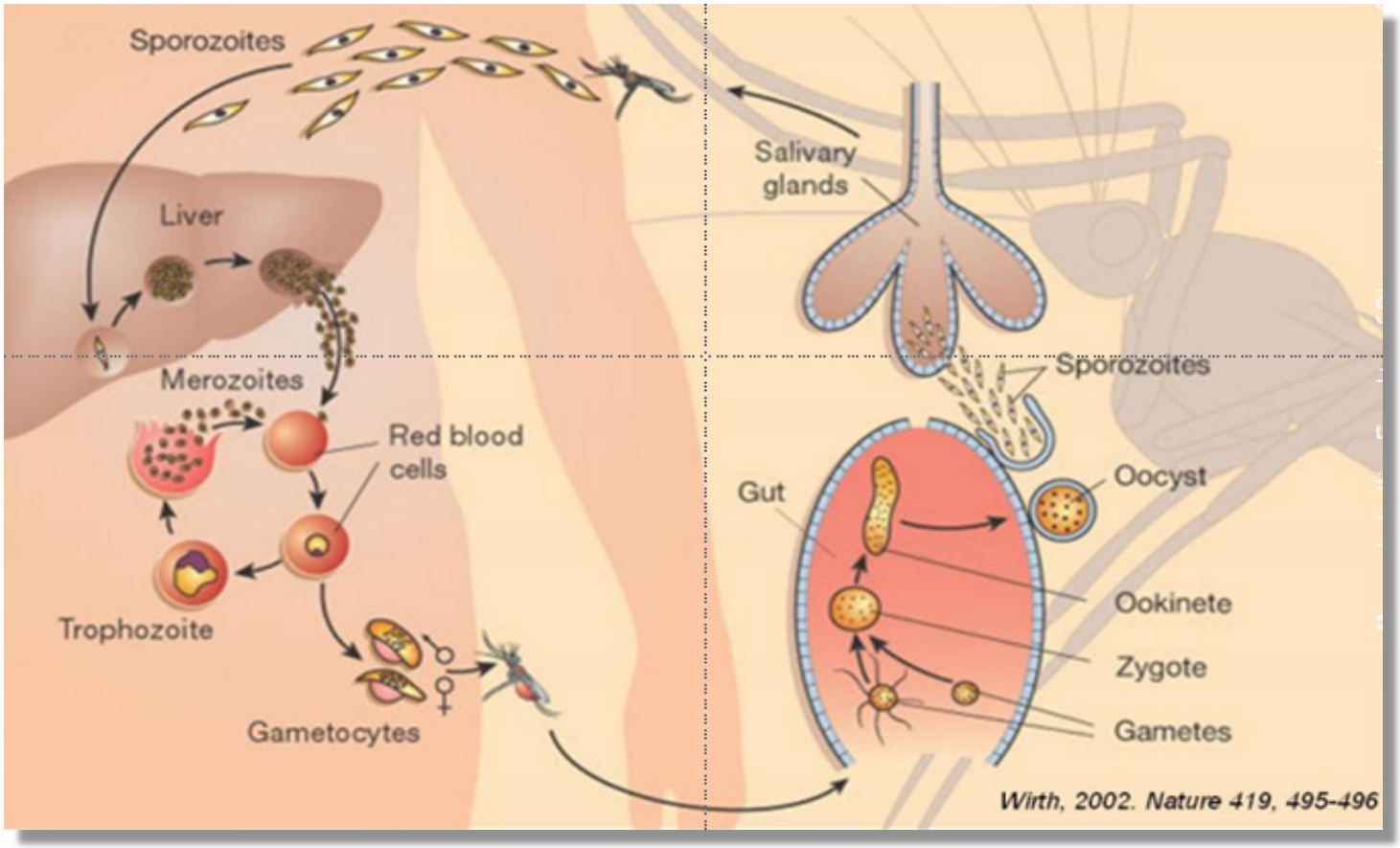
LUCE
BATTAGLIA
CONTRO
LA MALARIA

The parasite life cycle

Man

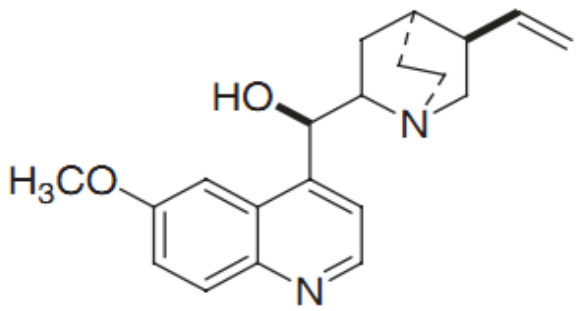
Parasite




The Lifecycle of Malaria
Human
Host

MOLECULES THAT CHANGED THE WORLD

K. C. Nicolaou



1 Quinine

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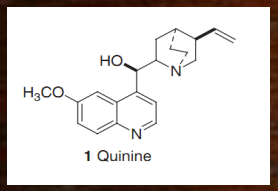
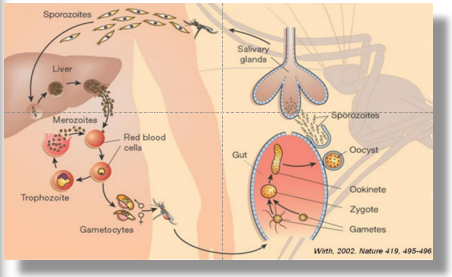
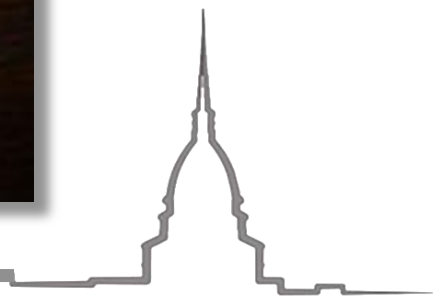
Battista Grassi (1854-1925)



1 Quinine





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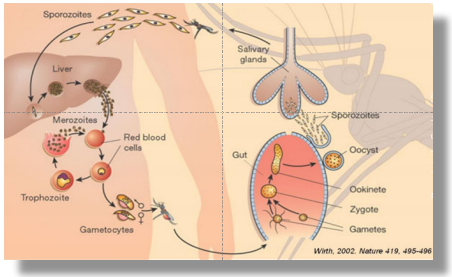
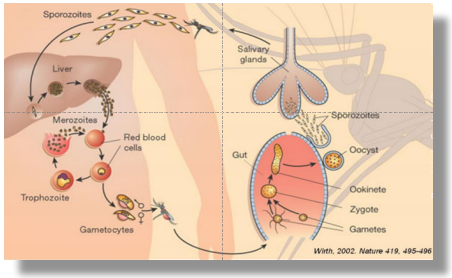
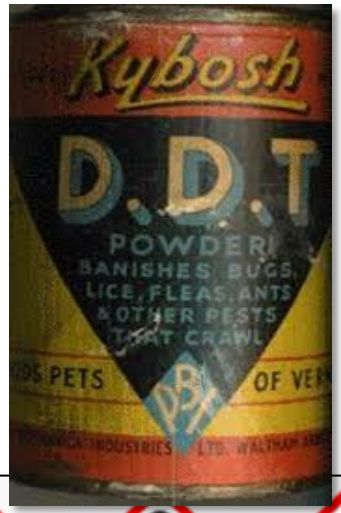
Development of a social model (early XX century)



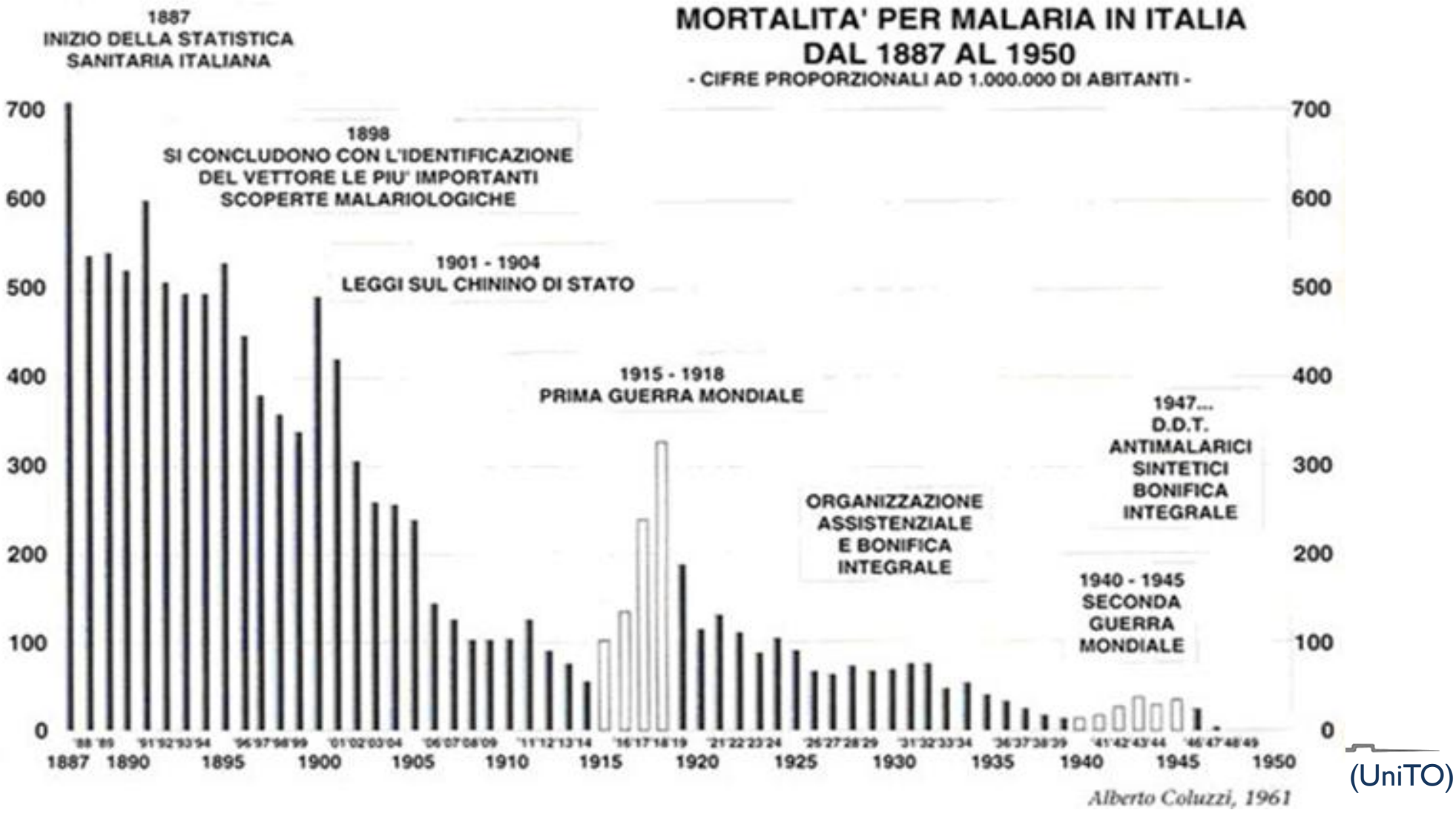
Chemistry gave an help?





Marco L. Lolli

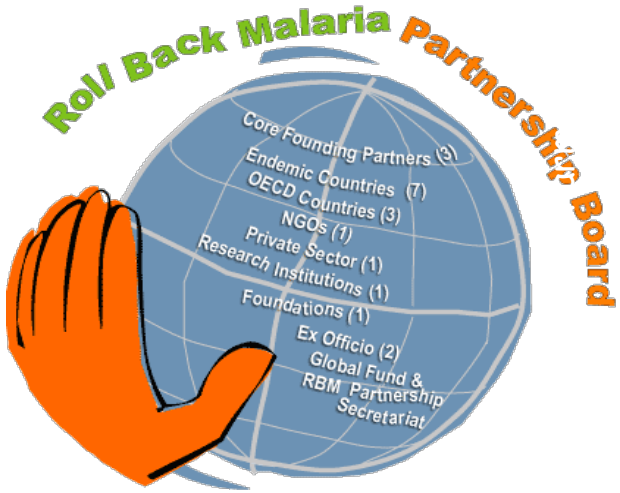
The battle is won!!!!!!



(UniTO)

Eradication of Malaria

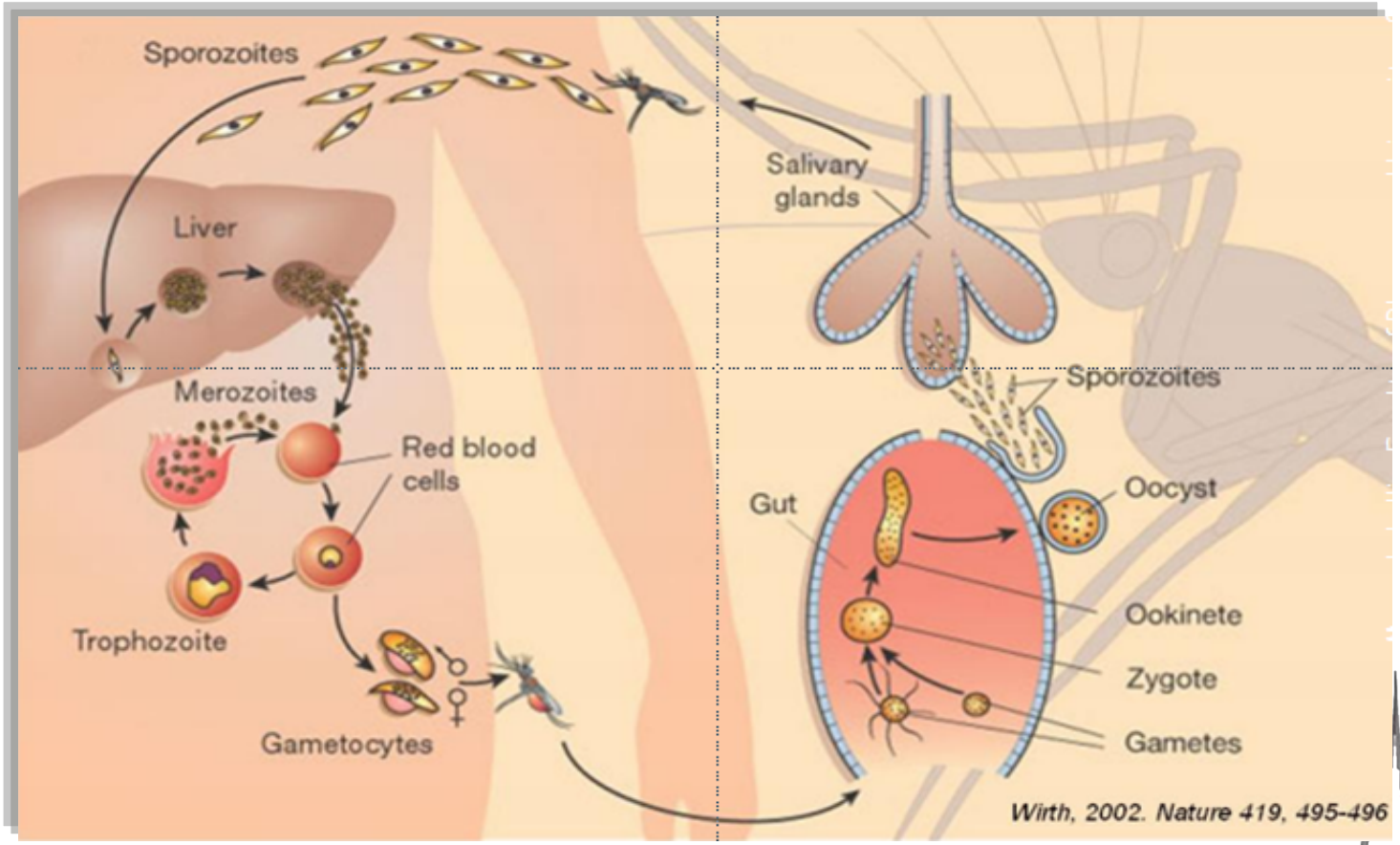
1934:	Hans Andersag at Bayer discovers chloroquine
1939:	Paul Müller at Geigy discovers DDT
1951:	Sardinia malaria free
1955:	WHA: goal of global eradication
1955-1969:	WHO uses DDT and chloroquine
50's:	DDT-resistance
1962-1970:	chloroquine-resistance
1955-1965:	expenditure of $ 1.4 billion
1962:	Italian last Malaria reported case
1969:	WHO back to malaria control
1975:	Europe free of malaria for first time in history



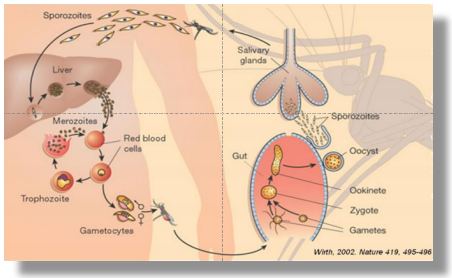
The parasite life cycle

Man

Parasite



The Italian school of *Malariatology*



The identification of the *anofele fly* role in the parasite circle

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Etiology of malaria

Discovery of the protozoan causative agents of malaria: *Plasmodium spp.*

Alphonse Laveran (1845-1922)

BULLETIN

DE

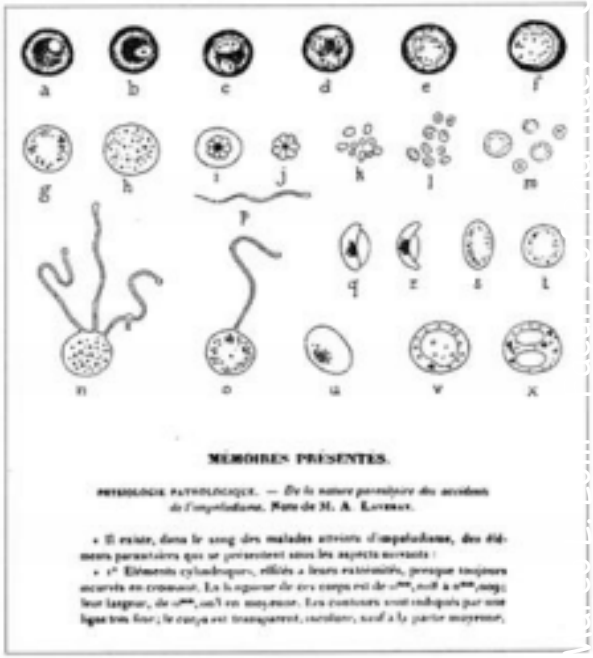
L'ACADÉMIE DE MÉDECINE

PARIS

M DCCC LXXX

J'ai l'honneur de présenter aussi à l'Académie, de la part de M. le docteur Laveran, professeur agrégé du Val-de-Grâce, actuellement médecin-major de 1re classe à l'hôpital militaire de Constantine, un manuscrit intitulé : *Note sur un nouveau parasite trouvé dans le sang de plusieurs malades atteints de fièvre palustre.*

Comme l'indique la figure qui y est jointe, l'auteur a rencontré dans le sang de ces malades divers éléments qui se présentent sous trois formes différentes : 1° Les uns allongés, cylindriques, un peu recourbés en croissant, offrant à leur partie moyenne une tache constituée par l'amas de granulations noirâtres ; 2° d'autres arrondis, d'un volume un peu inférieur à celui des globules rouges du sang, marqués aussi de granulations noires en forme de couronne, tantôt immobiles, tantôt mus par de longs tentacules qui s'insèrent sur leurs bords ; 3° des éléments encore arrondis, beaucoup plus gros que les précédents, dus sans doute au gonflement de ces derniers, à leur imbibition par le sérum.



MÉMOIRES PRÉSENTÉS.

PHYSIOLOGIE PATHOLOGIQUE. — *De la nature parasitaire des accidents de l'impaludisme.* Note de M. A. Laveran.

« Il existe, dans le sang des malades atteints d'impaludisme, des éléments parasitaires que se présentent sous les aspects suivants :

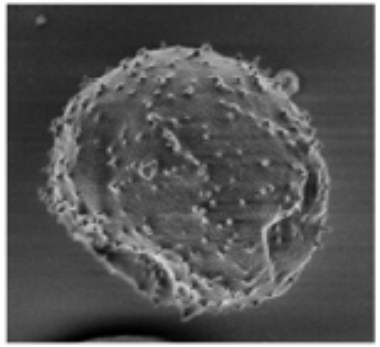
« 1° Éléments cylindroïques, effilés à leurs extrémités, presque toujours recourbés en croissant. La largeur de ces corps est de $\frac{1}{1000}$ mm à $\frac{1}{1200}$ mm ; leur longueur, de $\frac{1}{100}$ mm à $\frac{1}{200}$ mm en moyenne. Les contours sont indiqués par une ligne très fine ; le corps est transparent, incolore, sauf à la partie moyenne,

6 November 1880

Alphonse Laveran (1845-1922)

P. falciparum Remodels Both Outside and Inside of Erythrocyte

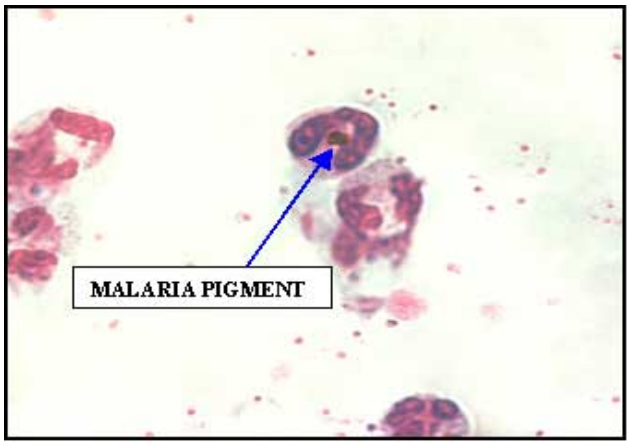
Normal erythrocyte

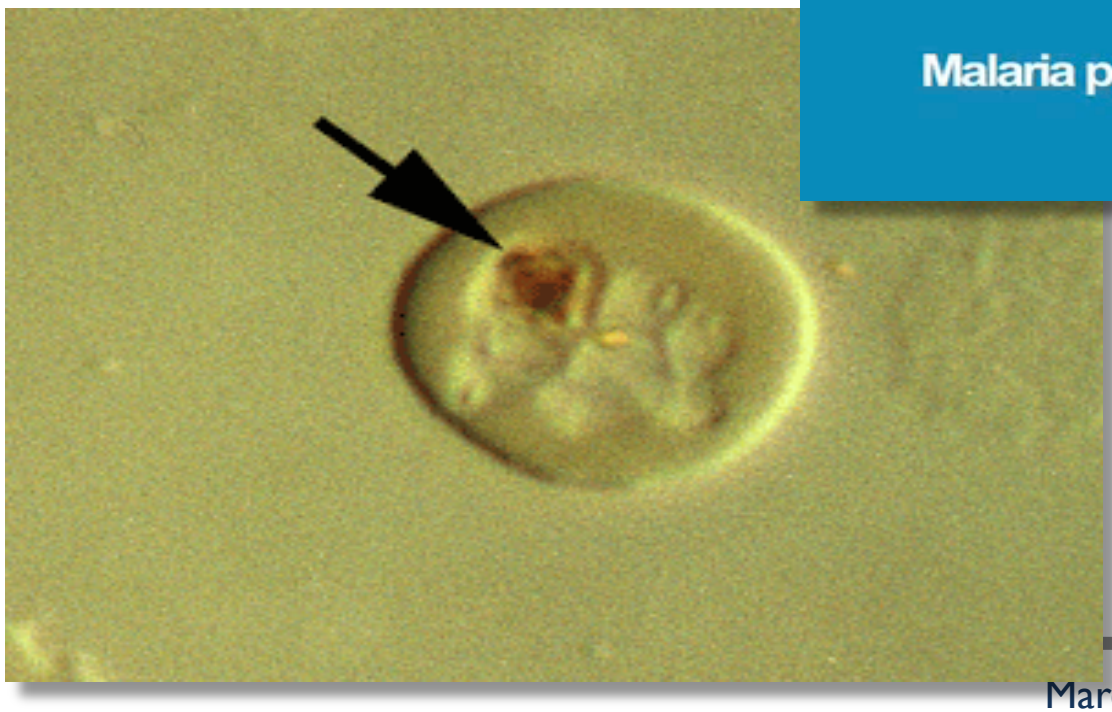


Infected erythrocyte

Infected erythrocyte

Plasmodium iron metabolism





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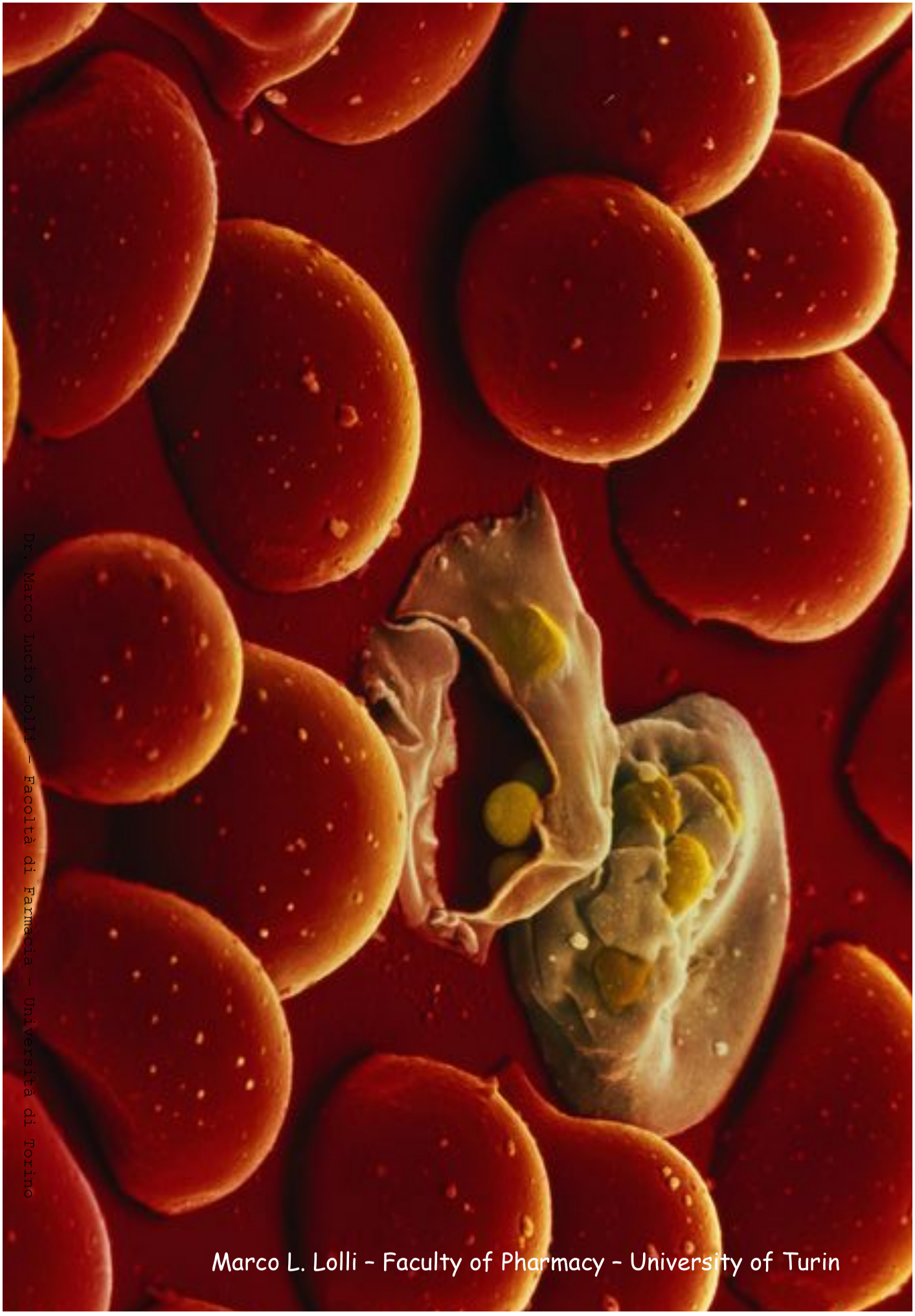
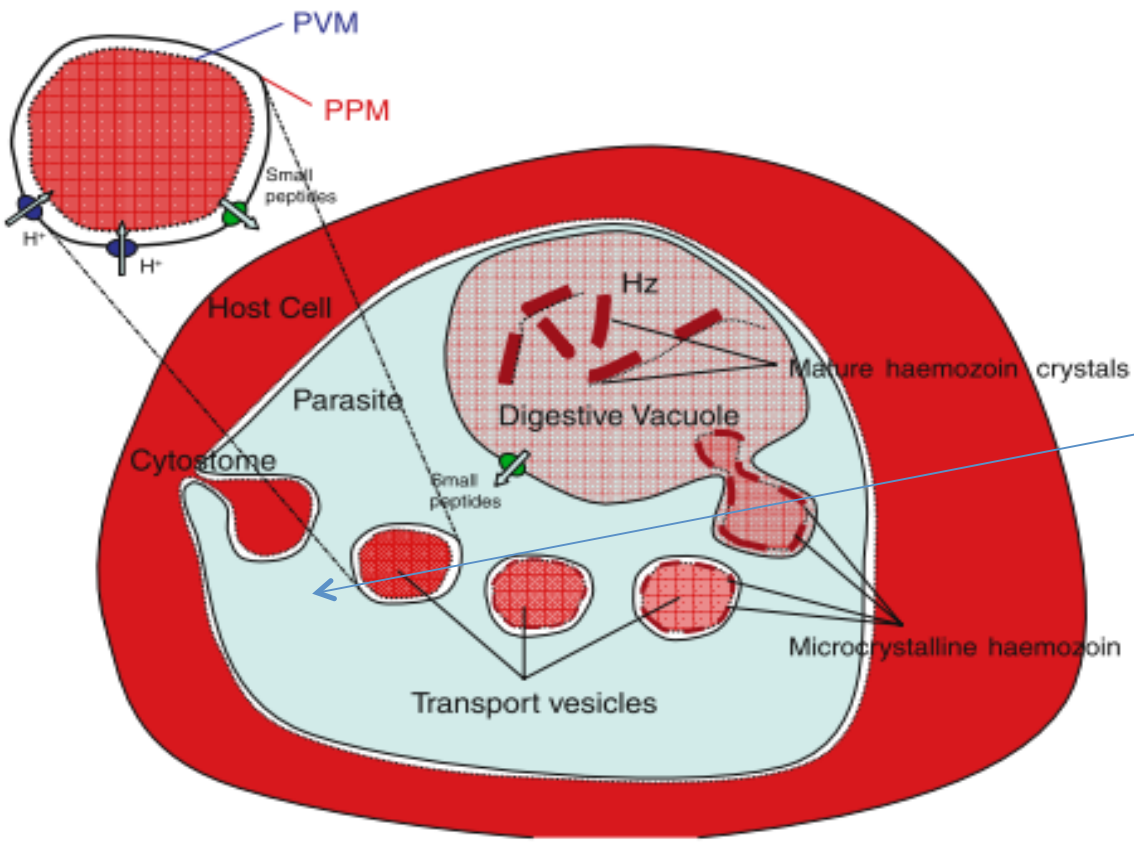

Dr. Marco Lucio Lolli – Facoltà di Farmacia – Università di Torino
Marco L. Lolli – Faculty of Pharmacy – University of Turin

Plasmodium iron metabolism



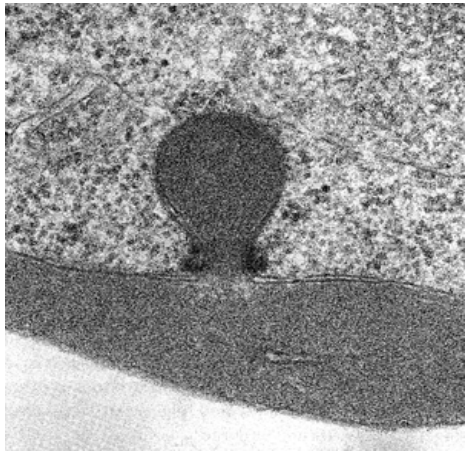
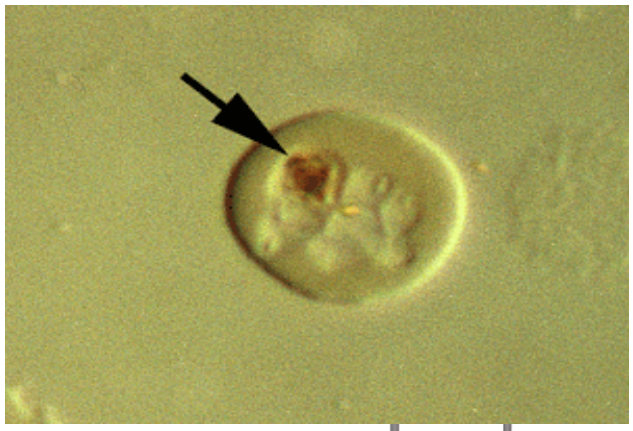
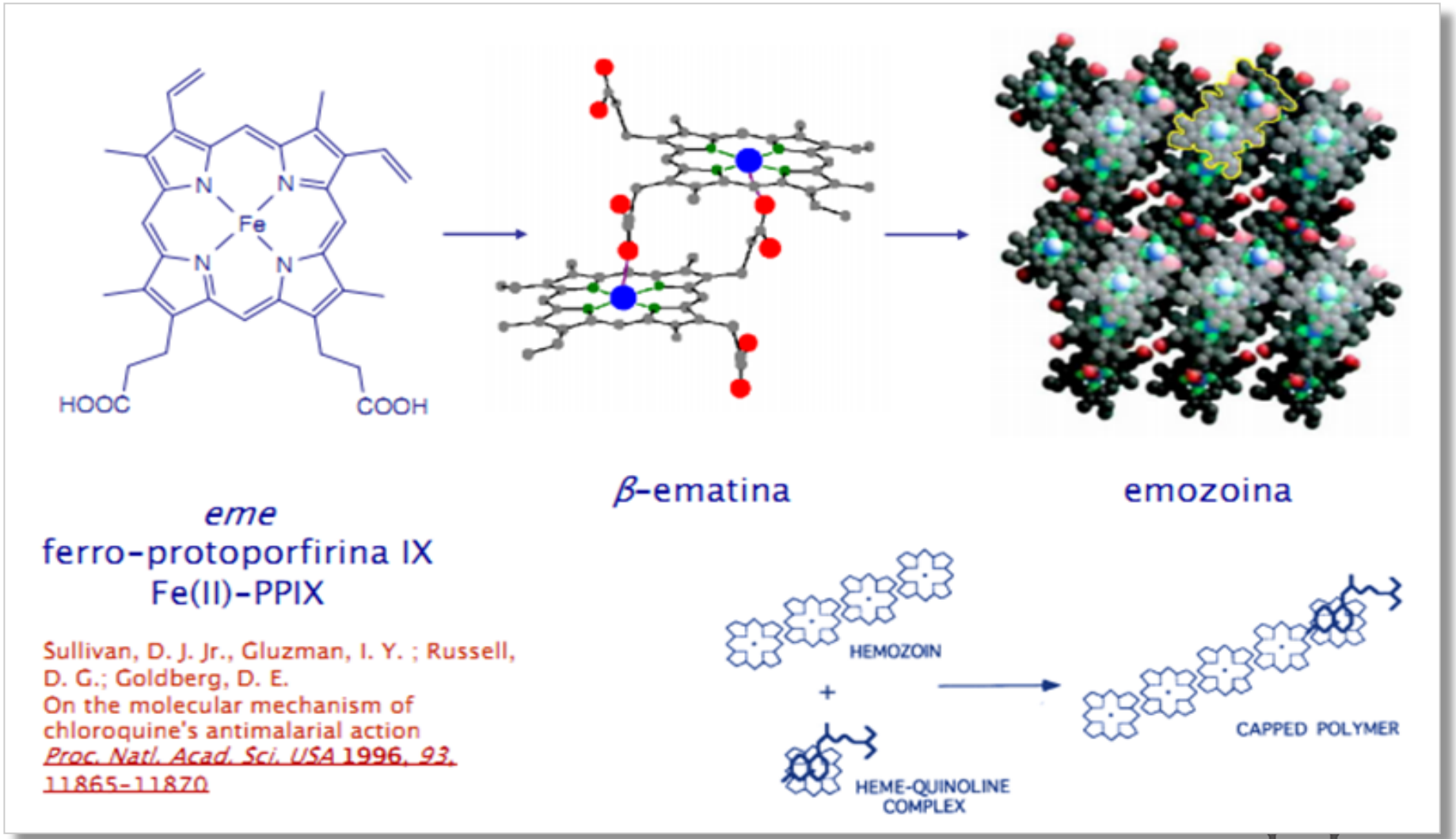


Fig. 1 Schematic showing the parasite feeding apparatus. Host cell cytoplasm is ingested by the cytostome and packaged in double membrane transport vesicles. The inner membrane is derived from the parasitophorous vacuolar membrane (*PVM*) and the outer membrane is derived from the parasite plasma membrane (*PPM*). Degradation of haemoglobin may start in the transport vesicles and be completed in the digestive vacuole. FP is deposited on the remains of the PVM which may act as a template for crystal formation. The vesicles fuse with the digestive vacuole and the immature crystals, residual membranes and haemoglobin are delivered to the digestive vacuole

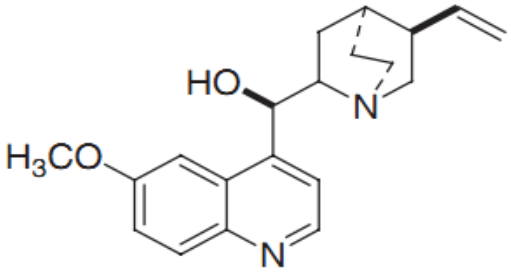


Hemozoin


N
N
Fe
N
N
HOOC
COOH
β-ematina
emozoina
eme
ferro-protoporfirina IX
Fe(II)-PPIX
Sullivan, D. J. Jr., Gluzman, I. Y. ; Russell, D. G.; Goldberg, D. E.
On the molecular mechanism of chloroquine's antimalarial action
Proc. Natl. Acad. Sci. USA 1996, 93, 11865-11870
HEMOZOIN
+
HEME-QUINOLINE COMPLEX
CAPPED POLYMER

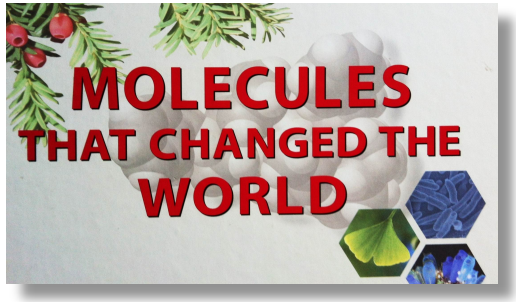
Cinchona officinalis (Rubiaceae)

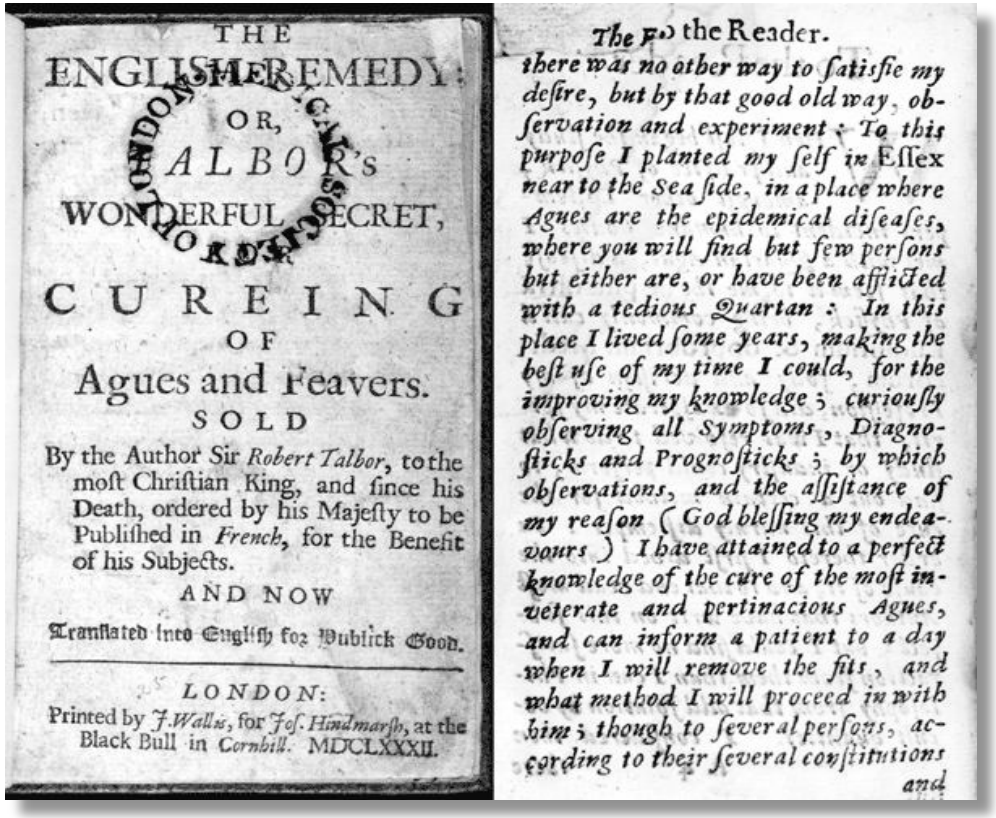
- Eastern slopes of the Andes.
- 1638: Countess Anna del Cinchon, wife of the Viceroy of Peru.
- 1640: **Jesuit's powder.**
- **Allowed European conquest of the tropics.**



1 Quinine

Cinchona officinalis (Rubiaceae)





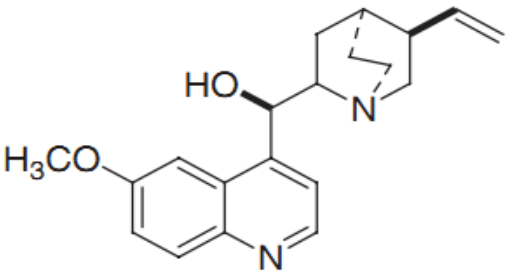
THE
ENGLISH REMEDY:
OR,
ALBOR's
WONDERFUL SECRET,
CUREING
OF
Agues and Feavers.
SOLD
By the Author Sir *Robert Talbor*, to the most Christian King, and since his Death, ordered by his Majesty to be Published in *French*, for the Benefit of his Subjects.
AND NOW
Translated into English for Publick Good.

LONDON:
Printed by *J. Wallis*, for *Jos. Hindmarsh*, at the Black Bull in *Cornhill*. MDCLXXXII.

The [illegible] the Reader.

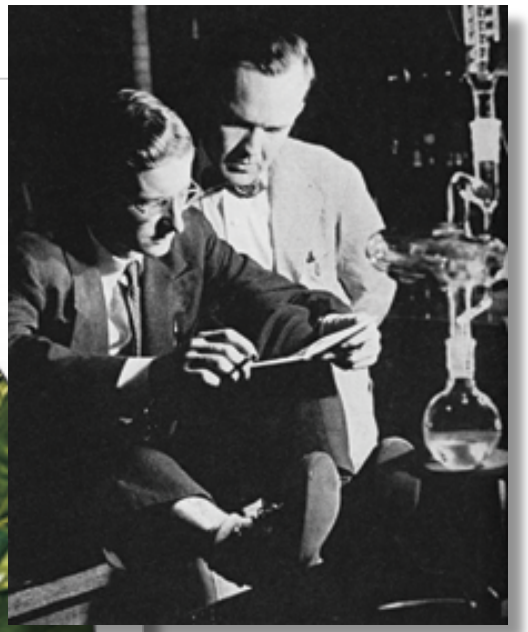
there was no other way to satisfie my desire, but by that good old way, observation and experiment: To this purpose I planted my self in Essex near to the Sea side, in a place where Agues are the epidemical diseases, where you will find but few persons but either are, or have been afflicted with a tedious Quartan: In this place I lived some years, making the best use of my time I could, for the improving my knowledge; curiously observing all Symptoms, Diagnosticks and Prognosticks; by which observations, and the assistance of my reason (God blessing my endeavours) I have attained to a perfect knowledge of the cure of the most inveterate and pertinacious Agues, and can inform a patient to a day when I will remove the fits, and what method I will proceed in with him; though to several persons, according to their several constitutions and

- 1672 Robert Talbor's remedy: an infusion of cinchona powder in white wine



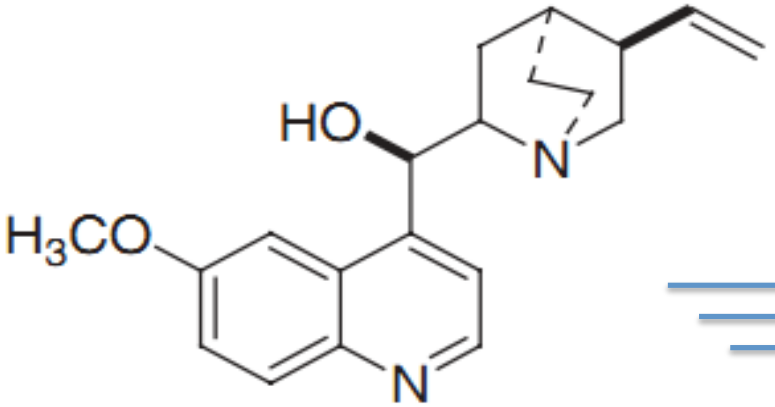
1 Quinine

Chemistry give an help!!

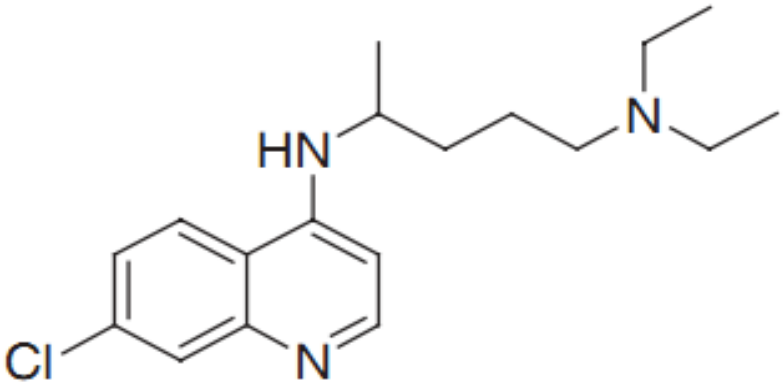


Woodward and the first **total synthesis** of Quinine (1944)

From quinine to synthetic analogues

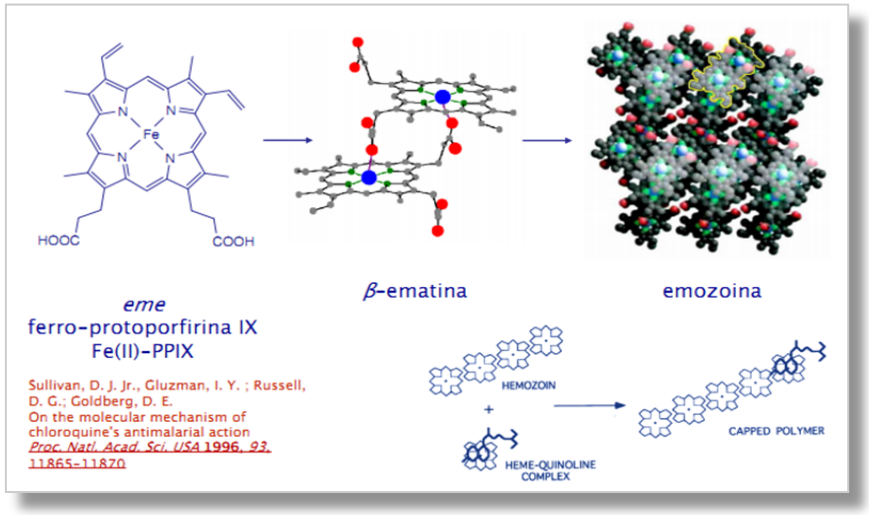


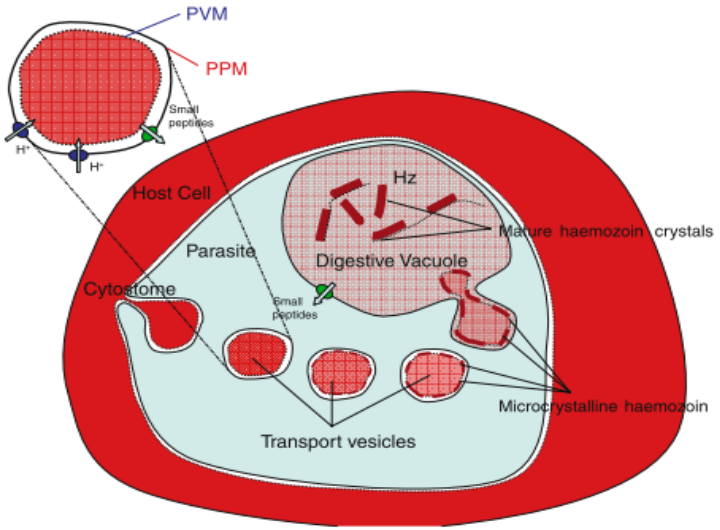
1 Quinine

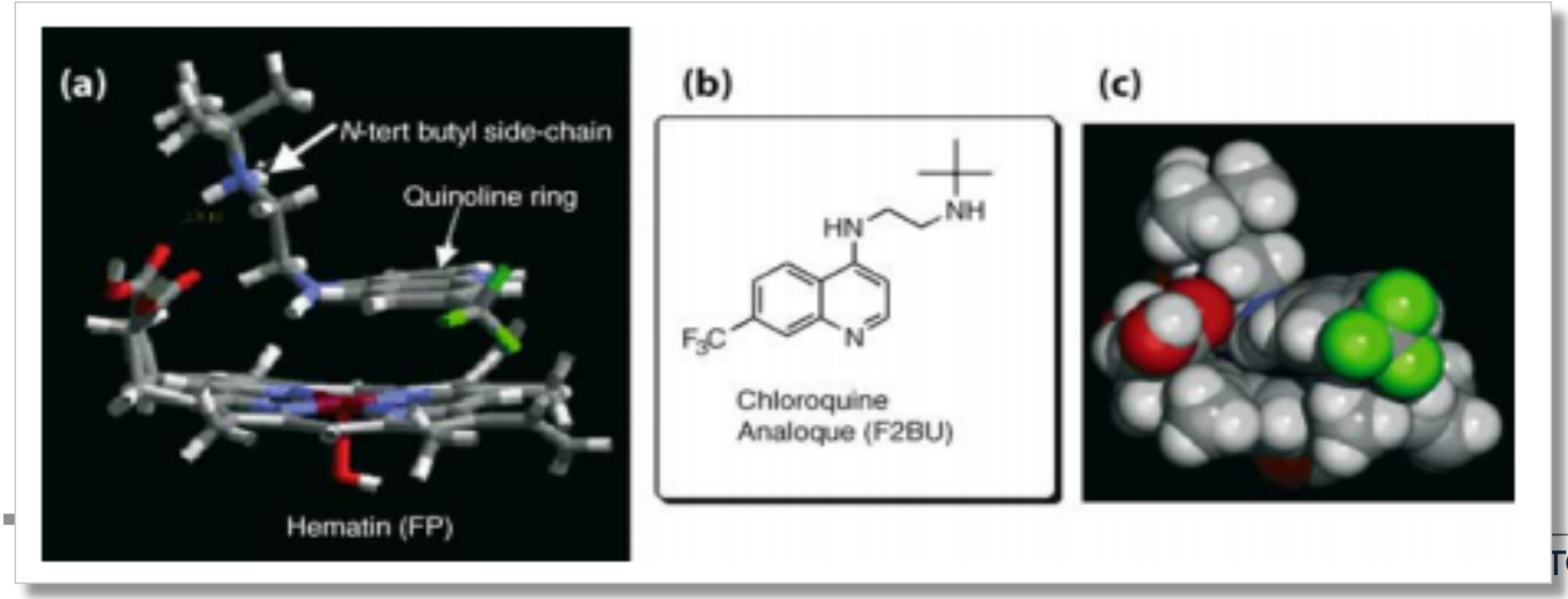


2 Chloroquine

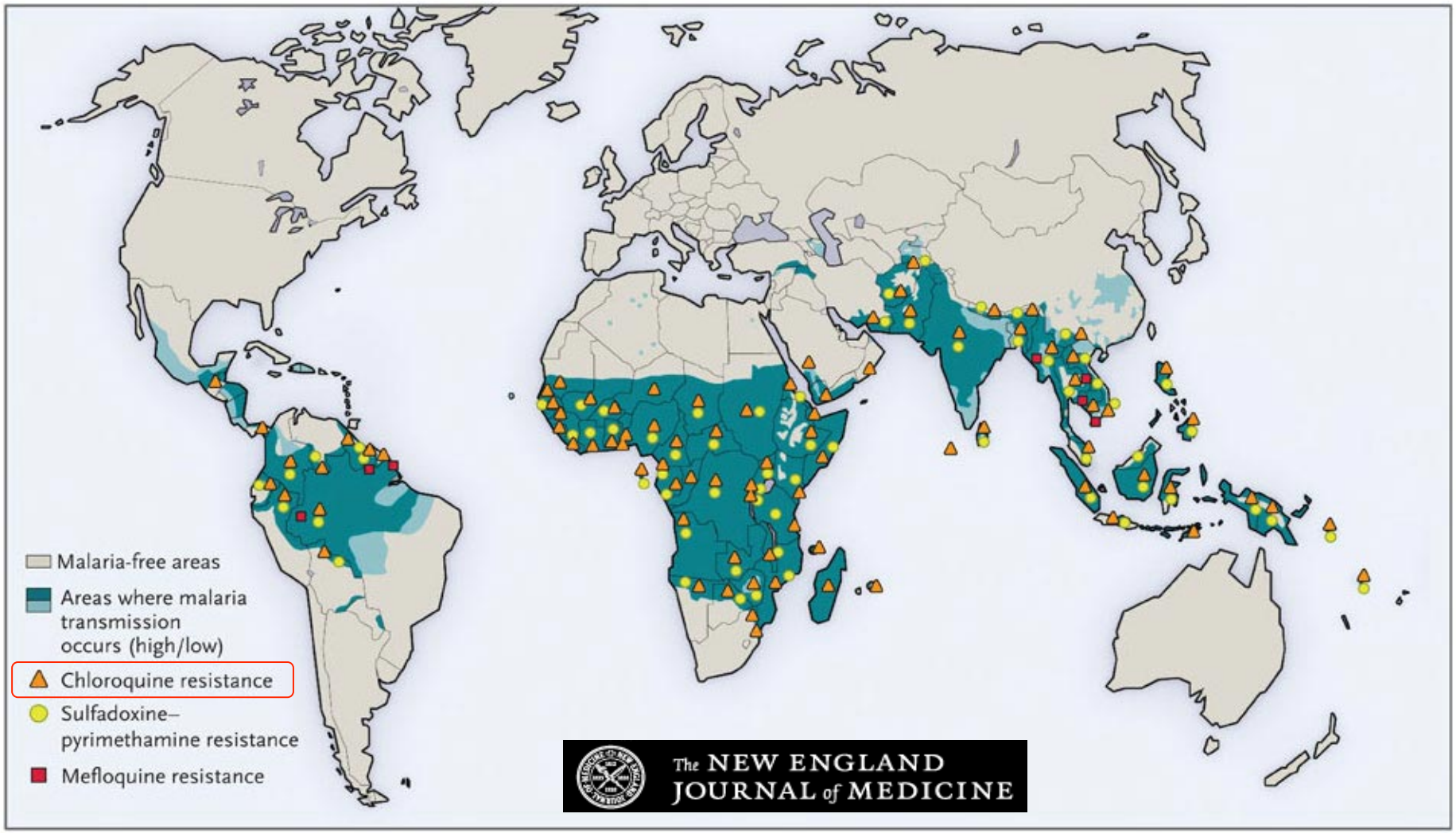
The effect of chloroquine on the Plasmodium iron metabolism





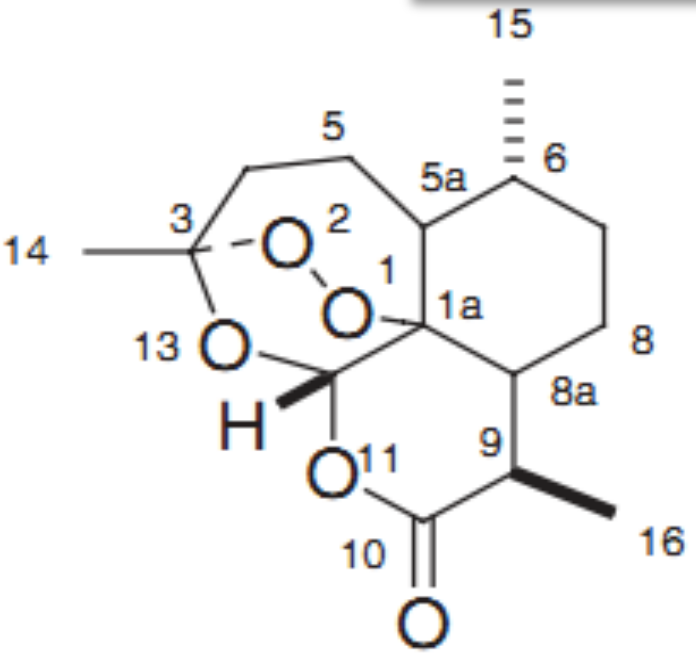


The Malaria problem



2008; 358: 2425-2428

Artemisia annua (qimghao)

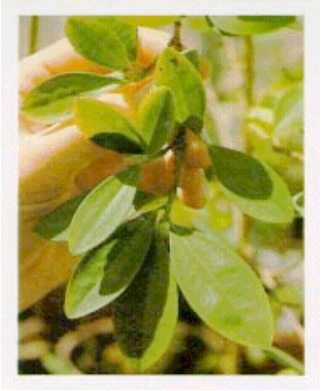


Artemisinin

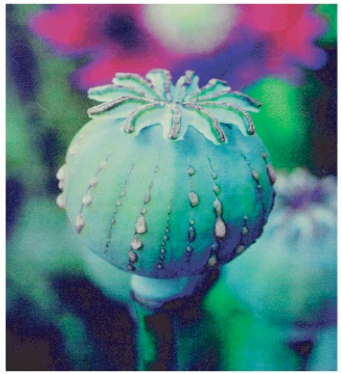
qimghaosu

The story of the Medicinal Chemistry:
the plants roles

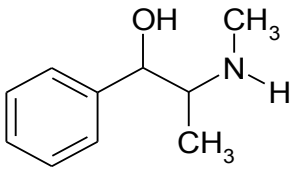
Ma huang



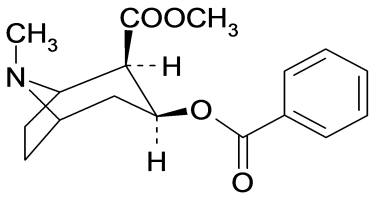
Erytroxylon coca



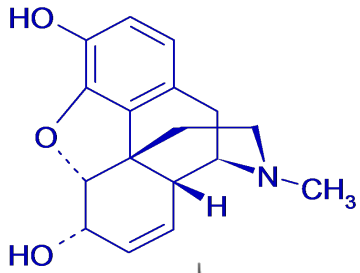
Papaver somniferum
(Oppio)



EFEDRINA
miscela di enantiomeri ERITRO



COCAINA

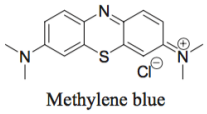


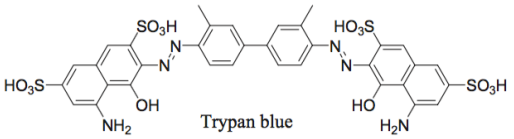
MORFINA

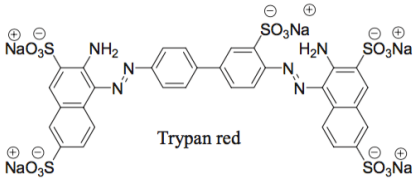
Broncodilatatore

Centrali

Paul Ehrlich:
the modern drug discovery







Chemoreceptors

- influence the interaction of cells with the chemicals around them to produced a biological effect.
- chemoreceptors of infectious organisms or cancer cells would be different from those of the host and that the differences could be exploited to produce a therapeutic benefit (the "**magic bullet**" theory).

and

- First example of **structure–activity relationships**
- First example of **Resistance** concept

Paul Ehrlich
(1854–1915)

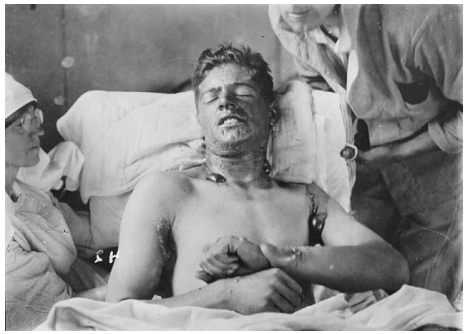
These concepts, along with his hypothesis that the chemical composition of drugs controlled their mode of action in an organism, formed the basis of modern chemotherapy.

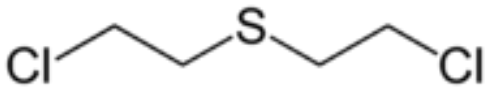
The role of serendipity

The birth of the chemio-theraphy: the Bari accident (Serendipity)

The air raid on Bari was an air attack by German bombers on Allied forces and shipping in Bari, Italy, on 2 December 1943 during World War II. In the attack, 105 German Junkers Ju 88 bombers, achieving complete surprise, bombed shipping and personnel operating in support of the Allied Italian campaign, sinking 27 cargo and transport ships and a schooner in Bari harbour.

SS John Harvey was a U.S. World War II Liberty ship carrying a secret cargo of **mustard gas**, whose sinking by German aircraft in December 1943 at the port of Bari in south Italy caused the single (and unintentional) release of chemical weapons in the course of the war by the Allies.





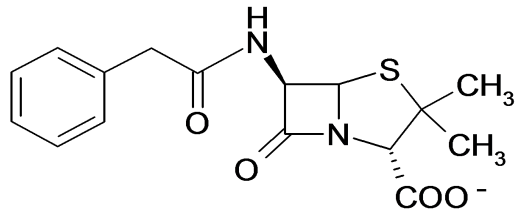
Mustard gas

White blood cells are capable of rapidly dividing which prompted the attention that this chemical agent could be useful in killing rapidly dividing cancer cells as well. The first clinical trial investigating the use of mustard gas was conducted by Louis Goodman and Alfred Gilman in 1942, just before the events at Bari. **As a consequence, the event at Bari enhanced the suspicion that the effect of mustard gas on blood cells could have medical use.**

Serendipity: always a good source of chemiodiverse drugs

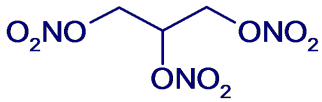
Menti preparate osservavano che…..:

✓ Nel 1928 Fleming osserva che una coltura batterica infettata da una colonia di funghi (*Penicillium*) presentava un'area in cui i batteri non crescevano.



BENZILPENICILLINA

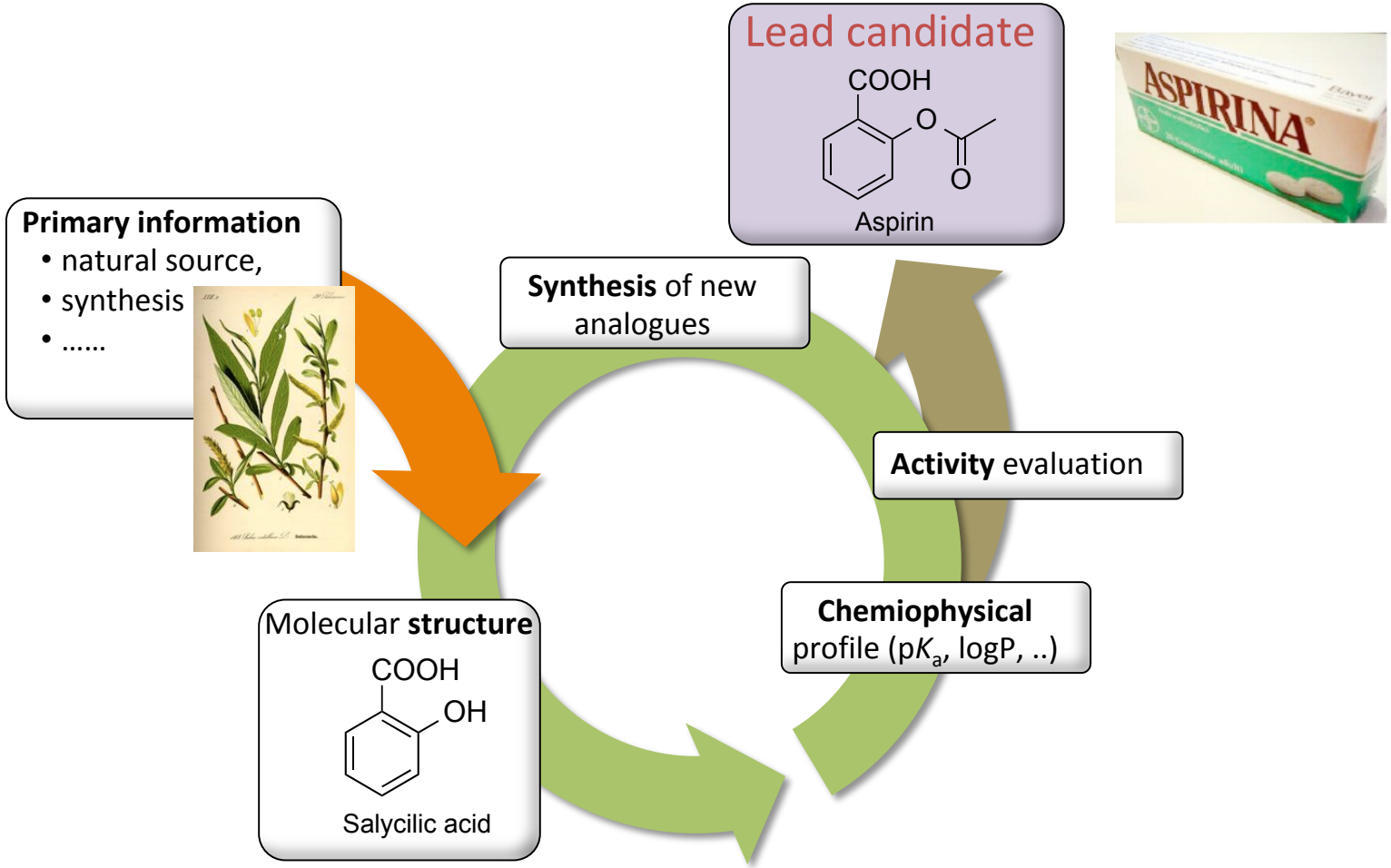
✓ Gli operai dell'industria degli esplosivi lamentavano forti e ripetuti mal di testa.



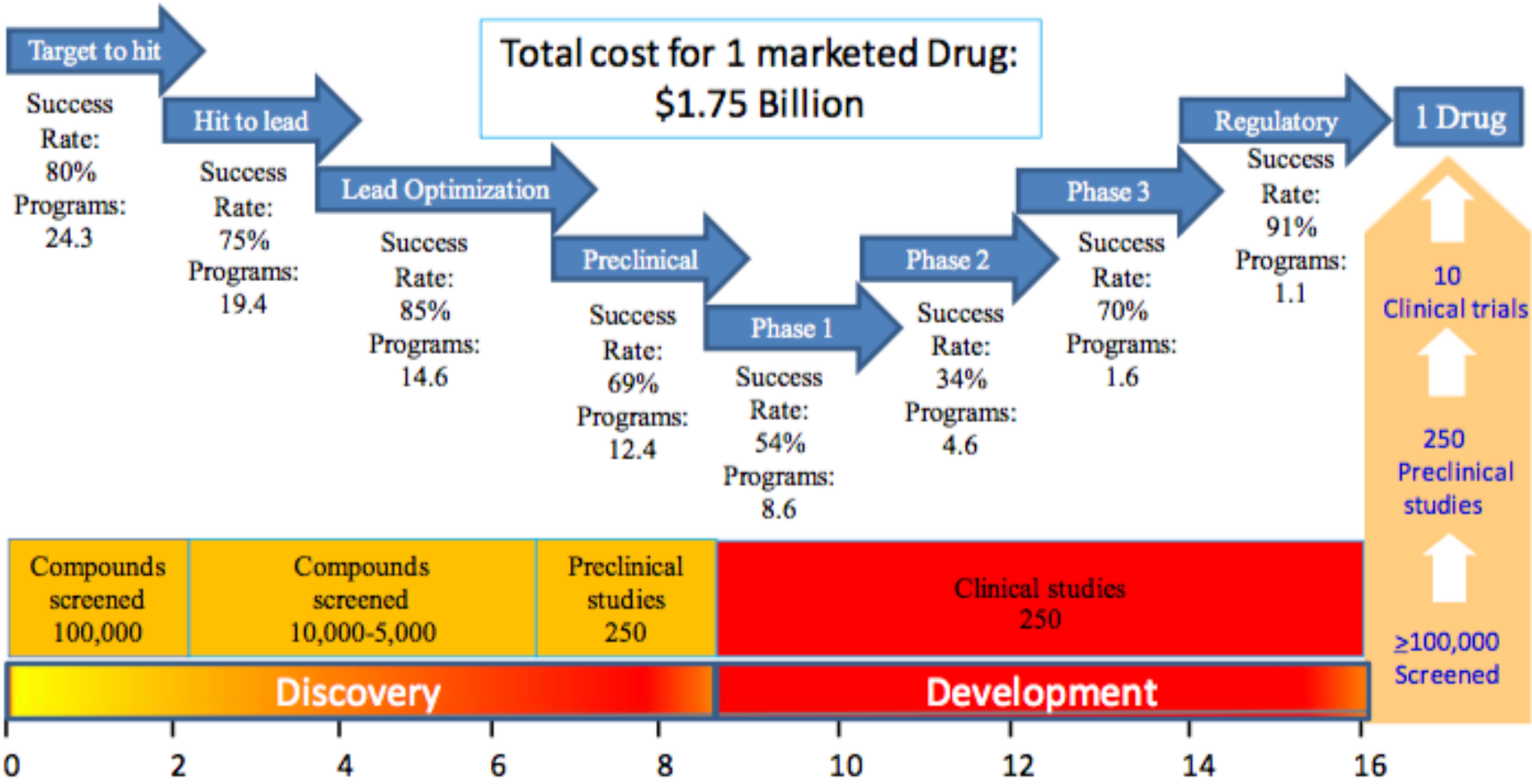
Glicerina trinitrato (GTN)

The Drug design modern process

Since then, the efficiently identify biologically active compounds and understand how they function, were developed.

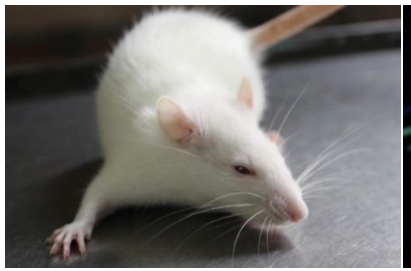


The Drug design modern process

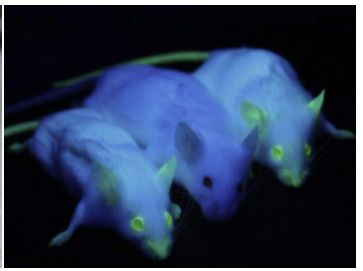


The drug design forced development of new technology in surrounding fields:

Animal modeling

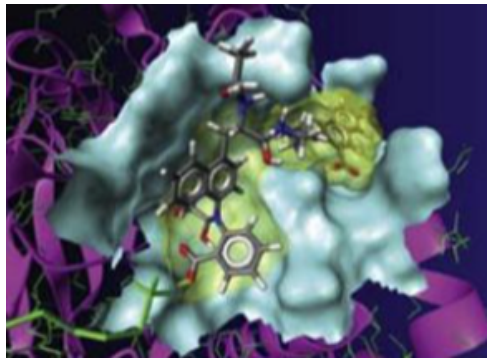


The Wistar rat



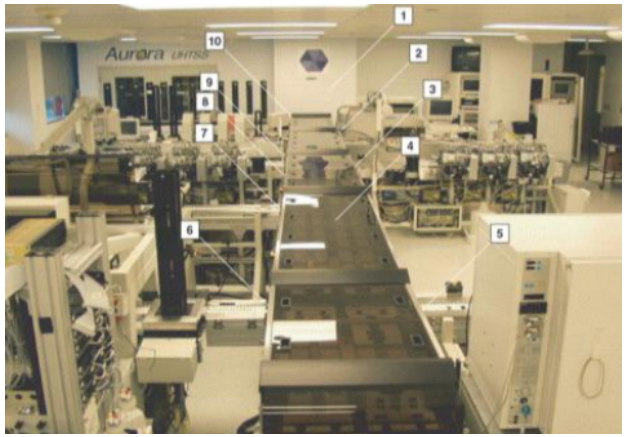
The knockout animal model

X-ray crystallography



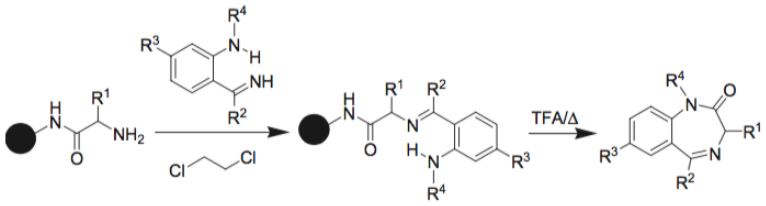
Molecular modeling

High throughput screening



Automated uHTS system at Bristol-Myers Squibb.

High throughput (combinatorial) chemistry

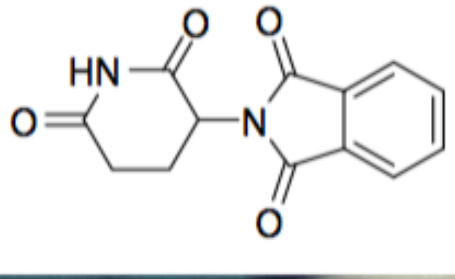


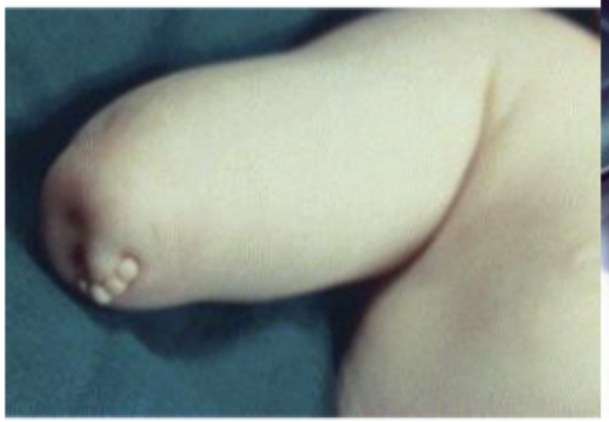
1992: the first solid phase synthesis by Ellman

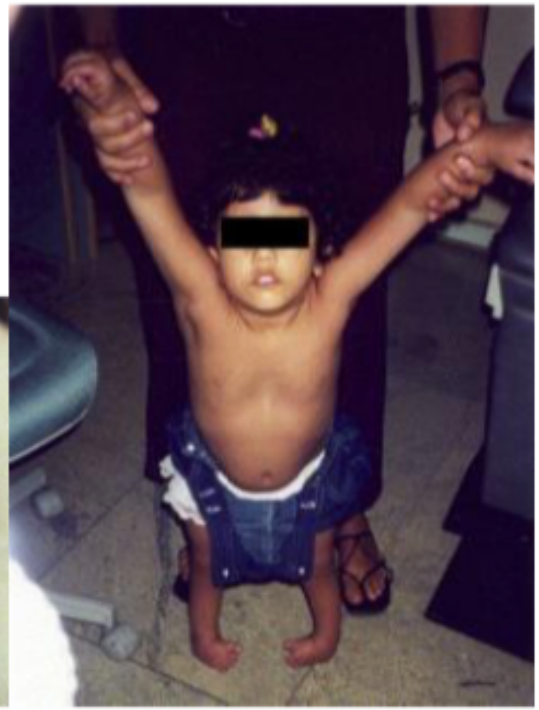
Biotechnology (recombinant DNA, …))

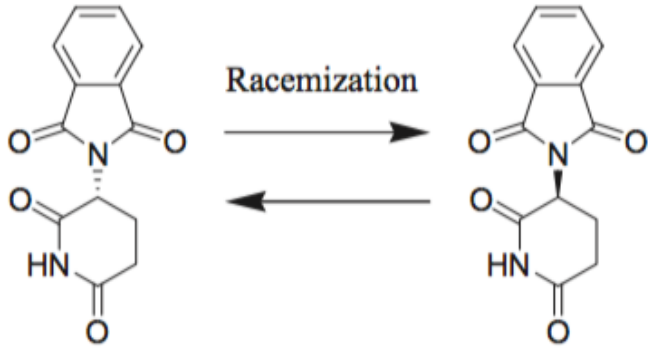
Genomics

Legislation and evolution of the Drug Safety









Thalidomide is not chi- rally stable in vivo. The (R) isomer (left) is readily converted to the (S) isomer (right). As a result, the pure (R) isomer is no safer than the originally marketed racemic material.

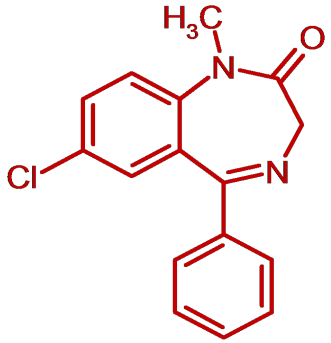
Thalidomide was brought to market in 1956 by Chemie Grünenthal GmbH as an over the counter treatment for morning sickness in pregnant women. By 1962, it had been withdrawn from markets across the globe as it had been definitively linked to severe birth defect in the children of woman that used it during their pregnancies.

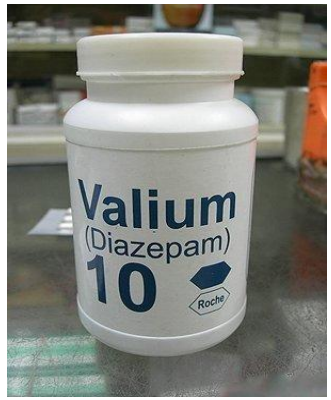
Nomi di farmaci

1. Nome chimico
2. Nome generico o denominazione comune internazionale (DCI); coniato dalla WHO/OMS (World Health Organization – Organizzazione Mondiale della Sanità)
3. Nome commerciale o registrato (brevettato); simbolo soprascritto ®, nome di fantasia; spesso registrato per il prodotto farmaceutico finito
4. Sigla

Esempio:

NOME BREVETTATO: VALIUM®
NOME GENERICO: DIAZEPAM





NOME CHIMICO: 7-cloro-1,3-diidro-1-metil-5-fenil-2H-1,4-benzodiazepin-2-one
SIGLA: 439-14-5 (numero di CAS); N05BA01 (ATC code = classificazione Anatomica Terapeutica Chimica una lettera: organo bersaglio; due cifre: gruppo terapeutico; una lettera: sottogruppo terapeutico-farmacologico; una lettera: classe chimica; due cifre: principio attivo)
N: sistema nervoso; 05: psicolettici; B: ansiolitici; A: derivati benzodiazepinici; 01: diazepam

NB. Nuovo principio attivo: brevetto (valido per 20-25 anni); può essere messo in commercio solo dalla ditta farmaceutica che lo ha brevettato (prezzi elevati). Scaduto il brevetto: farmaco generico, qualsiasi ditta può sintetizzarlo e metterlo in commercio (prezzi inferiori)

Classificazione Anatomica, Terapeutica e Chimica (ATC)

Elaborata nel 1976 dal centro di Documentazione sul Farmaco di Uppsala (Svezia) e adottata dal WHO/OMS

Primo livello ATC

A apparato gastrointestinale e metabolismo
B sangue ed organi ematopoietici
C sistema cardiovascolare
D dermatologici
G sistema genito-urinario ed ormoni sessuali
H preparati ormonali sistemici esclusi ormoni sessuali
J antimicrobici generali per uso sistemico
L farmaci antineoplastici ed immunososppressori
M sistema muscolo-scheletrico
N sistema nervoso centrale
P antiparassitari, insetticidi e repellenti
R sistema respiratorio
S organi di senso
V vari

Una lettera	organo bersaglio
Due cifre	gruppo terapeutico
Una lettera	sottogruppo terapeutico
Una lettera	classe chimica
Due cifre	principio attivo o associazione

Il sistema di classificazione ATC è basato sul principio che ad ogni preparato farmaceutico è associato un solo codice. I farmaci devono essere classificati in rapporto al loro impiego terapeutico prevalente.
Un farmaco può essere impiegato per due o più indicazioni terapeutiche di uguale rilevanza e ciò porta a differenti alternative per la sua classificazione

⇓

G01AA05: Cloramfenicolo

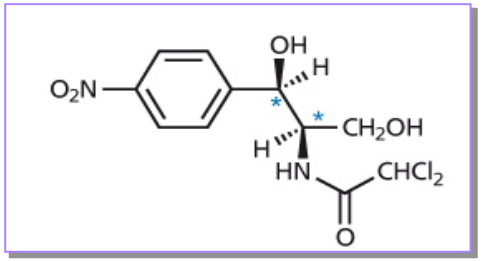
G01A Antinfettivi ed antisettici – ovuli vaginali

J01BA01: Cloramfenicolo

J01B amfenicoli – antibiotico sistemico

S01AA01: Cloramfenicolo

S01A Antinfettivi - colliri



Lezione #2 - Temi trattati (Conoscenza)

- Medicinal Chemistry: contesto storico generale
- Concetti di
  - *Chemical space*
  - Chemiodiversità
  - Drug design process
  - Eritrocita (Globulo rosso)
  - *Quinina*
  - *Artemisina*
  - Resistenza farmacologica
  - Total synthesis
  - *Antibiotici betaLattamici* e relativa reattività
  - Esteri nitrici come NO-*prodrugs*
  - *Cocaina*
  - *Morfina*
  - *Serendipity*
  - *Talidomide*
  - Animal modeling
  - X-ray crystallography

Concetti di:

- Molecular modeling
- High throughput screening
- Combinatorial chemistry
- Biotechnology (recombinant DNA, …))
- Genomics
- Nomenclatura di Farmaci
- *Diazepan*
- Drug safety
- Gruppo farmacoforico