Chapter 1

Introduction

Malaria kills over one million people each year, most of whom are children under 5 years of age (1) Each year there are over 300 million clinical cases of malaria, that is five times as many as combined cases of TB, AIDS, measles and leprosy Malaria is responsible for one out of every four-childhood death in Africa (2).

Malaria is a very old disease. It probably originated in Africa and accompanied human migration to the Mediterranean shores, India and South East Asia. It is generally associated with the marshy areas, dump spaces, which are excellent places for mosquito breeding. The correlation of mosquitoes with malaria was first noted in the 19th century by Dr Ronald Ross (3). Further discoveries in 20th century made it clear that Malaria is a protozoal disease transmitted by the Anopheles mosquito, caused by minute parasitic protozoa of the genus *Plasmodium*, which infect human and insect hosts alternatively.

Human malaria is transmitted only by mosquitoes belonging to the genus *Anopheles* (2,3) Currently 422 species of *Anopheles* mosquitoes have been identified throughout the world, 70 of which transmit malaria. Of the 70 species that transmit malaria, only 40 are of major significance

Anopheles mosquitoes are most frequently found in tropical regions, but are also found in temperate climates and in the Arctic during summer (see Table 1). As a rule, they are not found at elevations above 2000-2500 meters (6500-8200 ft).

In order for eggs to develop, female mosquitoes require at least one, sometime two blood meals Male mosquitoes do not take a blood meal For the blood supply i.e ingestion to be a continuos process no clotting should occur, so the females inject the salivary fluid

Table 1. *Anopheles* species of importance in transmission of human malaria.

Region	**Description**	**Major Vectors**
North America	From Great Lakes to southern Mexico	*A. freeborni* *A. quadrimaculatus* *A. hermsi*
Central America	Southern Mexico, Caribbean islands, fringe of South American coast	*A. albimanus* *A. argyritarsis* *A. pseudopunctipennis* *A. aquasalis* *A. darlingi*
South America	South American continent	*A. pseudopunctipennis* *A. punctimacula* *A. albimanus* *A. albitaris* *A. aquasalis* *A. darlingi*
North Eurasia	Europe and west Asia, Arctic south excluding Mediterranean coast	*A. atroparvus*
Mediterranean	South coast of Europe, North coast of Africa	*A. atroparvus* *A. labranchiae* *A. sacharovi* *A. superpictus*
Africa-Arabia	Saharan Africa, North Arabian peninsula	*A. pharoensis* *A. sergentii*
Sub-Saharan Africa	South Arabian peninsula, Ethiopia Somalia, tropical Africa, Madagascar	*A. funestus* *A. arabiensis* *A. gambiae* *A. melas* *A. merus*
India-Iran	Northwest of the Persian Gulf, Indian subcontinent	*A. culicifacies* *A. fluviatilis*
Indochina	Indochinese peninsula	*A. dirus* *A. maculatus* *A. fluviatilis* *A. minimus*
Malaysia	Indonesia, Malaysian peninsula, Philippines, Timor	*A. campestris* *A. donaldi* *A. letifer* *A. aconitus* *A. balabacensis* *A. dirus* *A. flavirostris*

		A. leucosphyrus *A. maculatus* *A. sundaicus*
China	Korea, Taiwan, Japan, and the Coast of mainland China	*A. anthropophagus* *A. sinensis*
Australia-Pacific	Northern Australia, Papua New Guinea, islands west of 175° east latitude	*A. farauti type 1* *A. farauti type 2* *A. koliensis* *A. punctulatus*

containing an anti-coagulant. Along with this are injected a large number of sporozoites of *Plasmodium*

Plasmodium is a group of one-celled animal parasites that lives on the red cells in the blood of many birds, reptiles and mammals. There are approximately 156 named species of *Plasmodium* which infect various species of vertebrates. Four are known to infect humans. *P. falciparum*, *P vivax* , *P. ovale* and *P. malariae* (2).

1. ***Plasmodium falciparum***, which causes the malignant tertian malaria. It is the most dangerous form of malaria. This species can produce overwhelming parasitemia (maximum 40% of the total RBC) and fulminating infection in non-immune patients.

2. *Plasmodium* ***vivex***, which cause benign tertian malaria and produce milder clinical attack that those of *P. falciparum*. Infection by *P. vivax* show low mortality rate and are characterized by relapse that occur as long as two years after primary infection.

3 ***Plasmodium ovale*** causes a rare malarial infection with a periodicity and relapse similar to *P vivax*, but is milder and more rapidly cured.

4. ***Plasmodium malariae*** cause quarten malaria, an infection that is common to localized area of tropics

Selected characteristics of the four species that cause human malaria are given in Table 2

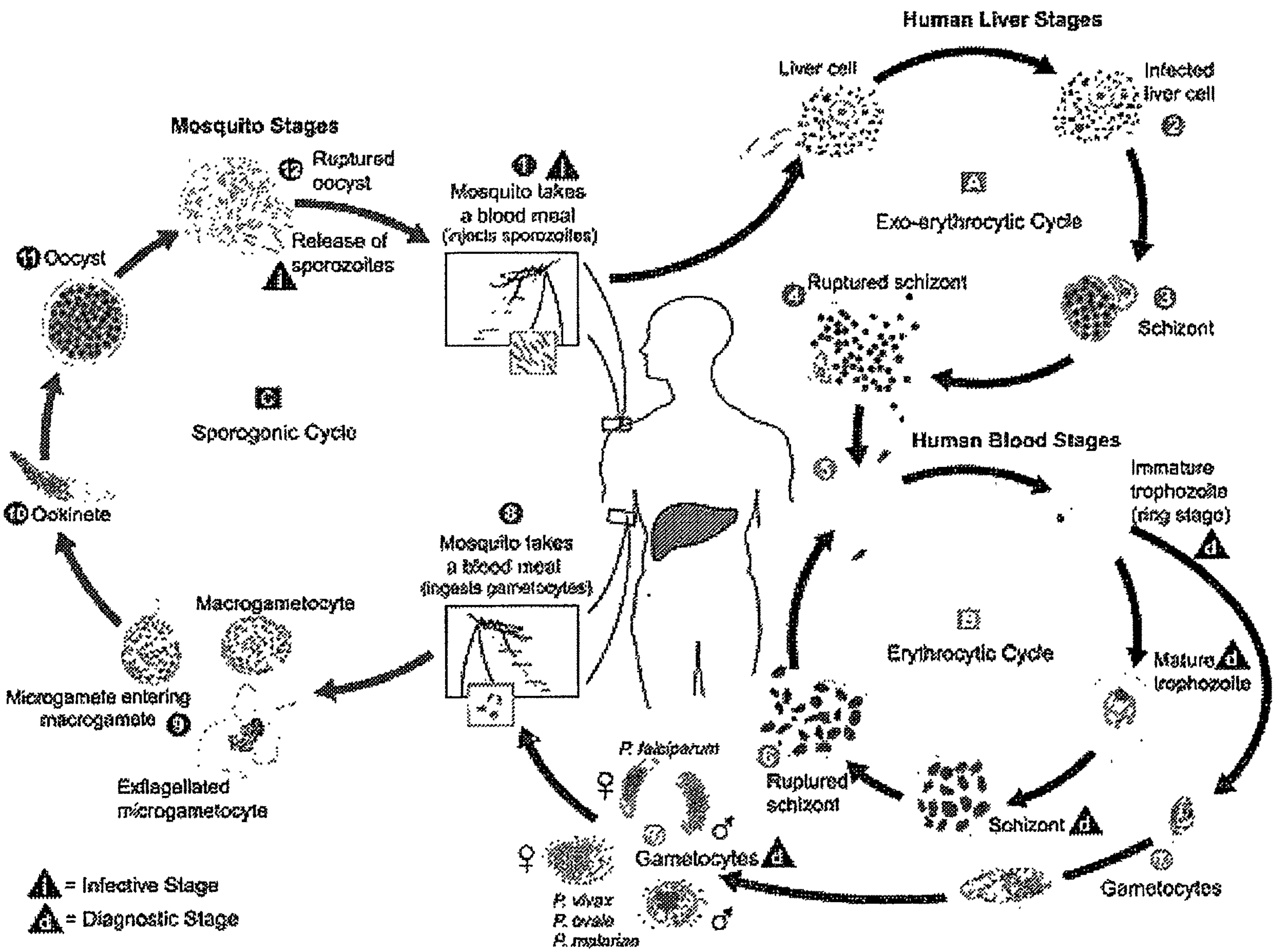
Table 2. Selected Characteristics of the Four Species of Human Malaria

	P. falciparum	P. vivax	P. ovale	P. malariae
Incubation days (range)	12 (9-14)	13 (12-17) or up to 6-12 months	17 (16-18) or longer	28 (18-40) or longer
Exoerythrocytic cycle (days)	5.5-7	6-8	9	12-16
No. of merozoites per liver cell	40,000	10,000	15,000	2,000
Erythrocytic cycle (hours)	48	42-48	49-50	72
Red blood cell preference	younger cells, but can invade cells of all ages	Reticulocytes	Reticulocytes	Older cells
Relapses	No	Yes	Yes	No
Fever periodicity (hours)	none	48	48	72
Febrile paroxysm length (hours)	16-36 or longer	8-12	8-12	8-10
Severity of primary attack	severe in non-immune		mild to severe	mild mild
Drug Resistance	++	+	—	—

Life Cycle

The life cycle of malarial parasite is complex (e.g. see Fig. 1) with developmental stages and corresponding symptoms differing according to the *Plasmodium* species involved As described earlier when a female *Anopheles* mosquito bites, with its salivary fluid there is an injection of thousands of sporozoites in the peripheral circulation. Following inoculation, the sporozoites disappear from the blood within 30 minutes. Many of them migrate to liver cells where they enter the cells and rest is destroyed by white blood cells (2) Sporozoites that enter the liver cells multiply asexually in a process called exoerythrocytic schizogony. This leads to production of a large number of merozoites which when the liver cells burst are released into the bloodstream. This process needs occurs 6 to 16 days after initial infection depending on the infecting *Plasmodium* species.

Merozoites from *P. falciparum* and *P. malariae* invade red blood cells (erythrocytes) and initiates the erythrocytic phase whereas the merozoites of *P. vivax* and *P. ovale* have two exoerythrocytic forms The primary type develops, causes liver cell rupture, and releases merozoites just as described for *P falciparum* and *P. malariae*. The other form, which develops concurrently, is known as the hypnozoite Sporozoites that enter liver cells differentiate into hypnozoites that remain dormant for weeks, months, or years. At some future time, the hypnozoites activate and undergo exoerythrocytic schizogony, forming a wave of merozoites that invade the blood and cause a delayed case or a clinical relapse. Released merozoites invades erythrocytes of different age and they further develop into trophozoites After a period of growth, the trophozoites divide and develop, eventually forming 8-24 merozoites in each red blood cell. When the parasite grows in the RBCs it consumes a large proportion of energy and the hemoglobin of RBCs. It utilizes the globin part as source of amino acids and sequesters the heme moiety When this process is


Human Liver Stages
Liver cell
Infected liver cell ②
Exo-erythrocytic Cycle
③ Schizont
④ Ruptured schizont
Human Blood Stages
⑤
Immature trophozoite (ring stage)
Mature trophozoite
Erythrocytic Cycle
Schizont
⑥ Ruptured schizont
⑦ Gametocytes
Gametocytes
P. falciparum
P. vivax
P. ovale
P. malariae
♀
♂
① Mosquito takes a blood meal (injects sporozoites)
⑧ Mosquito takes a blood meal (ingests gametocytes)
Mosquito Stages
⑫ Ruptured oocyst
Release of sporozoites
⑪ Oocyst
Sporogonic Cycle
⑩ Ookinete
Macrogametocyte
Microgamete entering macrogamete ⑨
Exflagellated microgametocyte
= Infective Stage
= Diagnostic Stage

complete, the host red blood cells rupture, releasing mature merozoites and a large amount of parasite debris and the hemozoin complexes which acts as pyrogens and is responsible for the symptoms associated with malaria.

The merozoites then invade fresh erythrocytes and another generation of parasites develops in the same manner. This process occurs repeatedly during the course of infection and is called eryrthrocytic schizogony. The length of this cycle differs according to the species of parasite, varying from 48 hours in vivax, ovale, and falciparum malaria, to 72 hours in *P. malariae* infections. Some merozoites differentiate into sexual forms (female macrogametocytes, male microgametocytes) and develop in invaded red blood cells.

Anopheles mosquitoes, which feed on infected hosts, ingest the RBCs containing the sexual forms The female macrogametocytes and male microgametocytes mature in the stomach of mosquito The fusion of the two results in the formation of a zygote that undergoes mitosis. The products of mitosis are ookinetes, which force themselves between the epithelial cells to the outer surface of the stomach, and form into small spheres called oocysts The oocysts enlarge as the nucleus divides, eventually rupturing and releasing thousands of motile sporozoites into the body cavity. The sporozoites migrate to the salivary glands, making the female mosquito infective. This phase known as sporogony, is complete in 8 to 35 days depending on species and environmental conditions

About 40% of the world area are under the malaria/ mosquito affected area (2). To combat malaria it requires or can be controlled via.

a) Preventing the mosquito growth / biting.

b) Preventing the growth of *Plasmodium*

a) Preventing the vector growth: a large number of insecticides have been used to control the growth of mosquitoes in various countries resulting in resistance in mosquitoes against some very potent insecticide such as DDT. The larva were also targeted using various larvacides and fishes which can eat larva. Still the best way to prevent malaria is preventing the biting. Insect repellents and insecticide (synthetic and natural) impregnated bednets are being successfully used in various countries of Africa (4)

b) Preventing the growth of *Plasmodium*: This is done by antimalarial drugs. Depending on the stages of *Plasmodium* at which they act they are classified in four groups.

(i) Blood Scizontocides
(ii) Tissue schizontocides or Hypnozoitocides
(iii) Gametocytocidal
(iv) Sporontocidal

Blood Schizontocides

These are used for clinical or suppressive cure. These important drugs act on asexual erythrocytic stage of malarial parasites to interrupt erythrocytic schizogony and thereby terminate clinical attack. Blood Schizontocides can be divided roughly into two groups according to their clinical efficiency and mechanism of action. First group includes the classical anti-malarial alkaloids, chloroquine, quinine, and related derivatives such as quinidine meflaquine and halofantrine. These weak bases concentrate in the erythrocytes infected with sensitive Plasmodia cause characteristic damage to the parasite. The second group includes pyrimethamine, chloroguanide and their derivatives that inhibit dihydrofolate reductase. It also includes sulfonamides and sulfones which interfere with dihydropteroic acid synthesis and thus with folate biosynthesis (a process that does not occur in mammals)

Tissue Schizontocides

These drugs are used for casual prophylaxis and are used to remove the tissue form of plasmodia within the liver either active or dormant Invasion of erythrocytes and further transmission of human malaria to mosquito is thereby prevented. Pyrimethamine and primaquine are the drugs that have casual prophylactic activity. Tissue Schizontocides used to prevent relapse. act on hepatic form of *Plasmodium vivax* and *Plasmodium ovale* that produce relapse after primary attack. Together with an appropriate blood Schizontocides these agents can achieve a radical cure of *Plasmodium vivax* and *Plasmodium ovale* infection

Gametecytocides

An agent of this type acts by destroying sexual erythrocytic forms of Plasmodia thereby preventing transmission of malaria to mosquito Primaquine has their type of activity, particularly against *Plasmodium falciparum*, chloroquine and quinine shows such activity against *Plasmodium vivax* and *Plasmodium malariae* but lack it against *Plasmodium falciparum*.

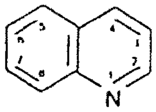
Sporontocides

These drugs ablate transmission of malaria by preventing or inhibiting formation of malarial oocytes and sporozoites in infected mosquito. Primaquine and chloroguanide are the anti malarials with this type of action.

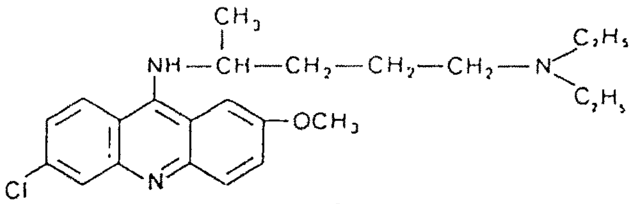
A large number of drugs are available around the world for treatment of malaria. These include doxycycline hyclate, halofantrine (Halfan7), mefloquine HCl (Lariam R), quinine, quinidine gluconate, atovaquone , chloroquine, hydroxychloroquine sulfate (Plaquenil R), pyrimethamine /sulfadoxine (Fansidar R), artemisinin, proguanil (paludrine), primaquine and WR 238605 (tafenoquine) (Fig. 2).

Choice of regimen is based on local cost and availability of antimalarial drugs, area of malaria acquisition (i e drug resistance pattern of p. *falciparum*), prior chemoprophylaxis, known allergies, concomitant illnesses other than malaria, age and pregnancy, likely patient compliance with therapy and risk of re-exposure to malaria after treatment Different regimens are available for the treatment of complicated and uncomplicated malaria

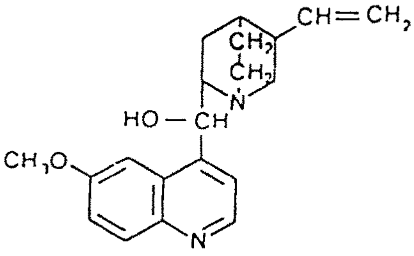
Chloroquine is the drug which is mostly used world wide, but the trend nowadays has gone back for natural antimalarials such as quinine because of the developing resistance to the chloroquine and its congeners As has been described earlier, at present, the only



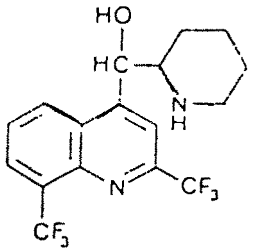
Quinoline ring



Quinacrine



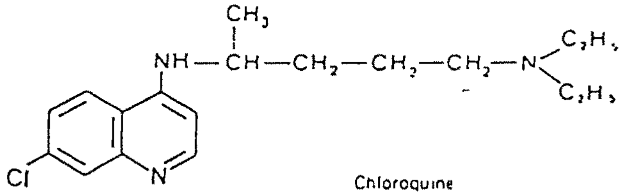
Quinine



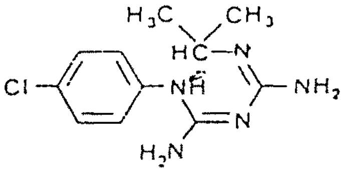
Mefloquine

4-Aminoquinolines

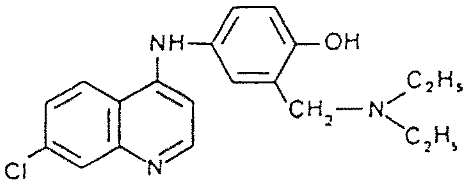
Dihydrofolate reductase inhibitors



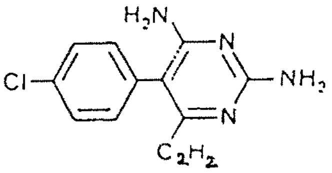
Chloroquine



Chloroguanide (proguanil)

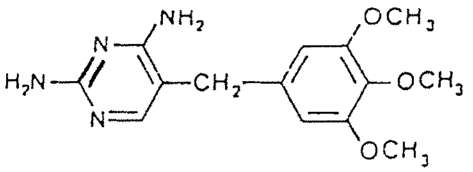


Amodiaquine

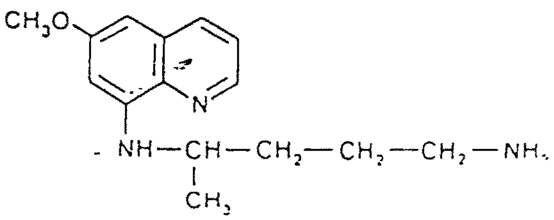


Pyrimethamine

8-Aminoquinoline

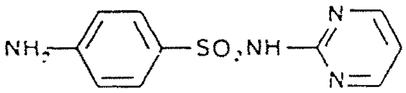


Tameroquine

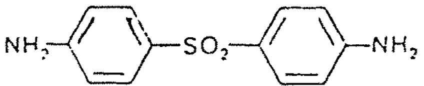


Primaquine

Sulfones and sulfonamides



Sulfadiazine



Dapsone
(diaminodiphenylsulfone DDS)

available tissue schizontocide is primaquine. Due to this reason, the present study is carried out with the three main anti malarial drugs Chloroquine, Primaquine and Quinine. The detailed history, mechanism of the anti malarial action, fate, absorption, excretion, toxicity and side effects of these three drugs are discussed below. Brief descriptions of other available drugs are also given later

Chloroquine

History

Chloroquine is one of a large series of 4-amino quinolines investigated as a part of the extensive cooperative program of anti malarial research in the U.S. during World War II. The objective was to discover more effective and less toxic suppressive agent than quinacrine, an acridine derivative abandoned for anti malarial chemotherapy because of its toxicity and inability to cure *vivax* malaria or to act as a casual prophylactic. Although the 4-amino quinoline had been described as potential antimalarial by Russian investigators, serious attention was not paid to this chemical class until the French reported that one derivative was well tolerated and had high activity in human malaria (2,3)

Mechanism of Action and Pharmacological Effects

Although Chloroquine was developed primarily as an anti malarial agent it possess several other pharmacological properties. It is used to treat extra-intestinal amoebiasis (2). The anti-inflammatory effects of Chloroquine are well known. The drug has been used occasionally in the treatment of rheumatoid arthritis and is more frequently used for discoid lupus erythromatosus; its efficiency in the latter condition is controversial (5).

Chloroquine has been employed with success to treat porphyria cutanea tarda, solar utricaria and polymorphus light eruption. Treatment of this condition requires much larger doses than are used for malaria (6,7). Recently role in Graft-versus-host disease (GVHD) has been reported for chloroquine and hydroxychloroquine (8)

Antimalarial action

Chloroquine given in massive doses exerts no significant activity on erythrocytic tissue stages of *Plasmodia* The drug is thus not a casual prophylactic agent and does not prevent the establishment of infection. However, it is highly effective against the asexual erythrocytic form of *Plasmodium vivax*. It is superior to quinine in suppressing *vivax* malaria In the acute malarial attack, Chloroquine rapidly controls clinical symptoms and parasitimia. Most patients become completely a fibril within 24 –48 hrs. After receiving therapeutic dose thick smears of peripheral blood are generally negative for parasites for 48- 72 hrs.

Mechanism of Anti-malarial action (9-11)

The primary mechanism of action of Chloroquine, quinine and related blood schizontocides remains obscure. A 1960's postulate states that Chloroquine acts by interfering with nucleic acid synthesis possibly by interacting with plasmodial DNA. A more recent hypothesis is that ferri-protoporphyrine IX (hemin) released during degradation of hemoglobin and parasite's erythrocyte serves as a receptor for Chloroquine and other anti malarial alkaloids and that the resulting drug-hemin complex has deleterious effect on the membranous structure of the *Plasmodia*. Another view is that the anti malarial alkaloids (blood schizontocides) concentrate in and rise the pH of acidic vesicles within sensitive malarial parasite. There they interfere with lysosomal degradation of hemoglobin and cause histological abnormalities characteristic of the particular drug class. .Chloroquine also inhibited the heme ploymerase of *Plasmodia* (12,13).

Absorption, Fate and Excretion (14)

Chloroquine is absorbed well (90%) from the GIT and is absorbed rapidly from intra-muscular or sub-cutanous sites. The drug is mainly metabolized in the liver, spleen, kidney and lungs and also in melanin contaning tissue and to lesser extent in brain and spinal cord. Chloroquine undergoes appreciable biotransformation. The major metabolite monodesenthylchloroquine has anti malarial activity and reaches plasma concentrations that approximate 20 – 35 % of those of the parent compound. The renal clearance of Chloroquine is about half of its total systemic clearance. Uncharged Chloroquine and its major metabolites account for over 50% and 20% of the urinary drug products respectively

Toxicity and Side effects

The amount of Chloroquine employed for oral therapy of the acute malarial attack may cause GI upset, pruritus, mild and transient headache and visual disturbance. Prolonged medication dose for suppressive purpose causes significant untoward effects and only rarely must the drug be discontinued because of intolerance. All symptoms readily disappear when the drug is withheld. Chloroquine may cause discoloration of nail buds and mucus membranes (2).

Chloroquine is reported to inhibit eel AChE (15), microsomal AChE and Na+, K+, ATPase (16). Chloroquine decreases the recruitment of insulin receptor (17). It is a potent inhibitor of succinate dehydrogenase (18), glutamate dehydrogenase (19) and NADH dehydrogenase (20). Chloroquine also decreases cholesterol content, cytochrome content and the respiration control ratio (20). Chloroquine stimulated various ATPase and PNPPase (21) and the effect was reversed at high concentrations. Chloroquine also induces acute structural alteration of choroid plexus epithelial cells, epidermal cells, and epiplexus cells (22). Cardiodepressant action of chloroquine are attributed in part to the alterations in Ca^{2+} accumulating ability of the mitochondria membrane (23)

Primaquine

History

In 1891 Ehrlich discovered that methylene blue exhibited weak plasmodial activity, later it was shown that 8-aminoquinolin had weak schizontocidal activity and also that the slight anti malarial potency of methylene blue could be intensified by substitution of alkylaminoalkyl group for one of the n-methyl group of the dye.

Pharmacological Effects

The great clinical value of primaquine lies in the radically curative treatment of *vivax* and *ovale* malaria and in unusual situation where chloroquine treatment has proven unsatisfactory In its use as supplement to suppression with chloroquine, primaquine is also highly active against the primary exo-erythrocytic form of *Plasmodium falciparum*, but this activity is of relatively little practical value. The activity of primaquine against erythrocytic stage of *Plasmodium vivax* has little clinical application. The drug is almost completely inactive against the asexual form of *Plasmodium falciparum*, and it is for this reason that primaquine is almost always used in conjugation with a blood schizontocides. The 8-amino quinolines exert a marked gametocidal effect against all four species of *Plasmodium* that infect man especially *Plasmodium falciparum*

Mechanism of anti-malarial action

Little is known about the action of 8-aminoquinolines especially why they are far more active against tissue form of Plasmodia. Primaquine may be converted to electrophiles that act as oxidation-reduction mediators. Such activity could contribute to antimalarial effects by generating reactive oxygen species or interfering with electron transport (2) There is some evidence that primaquine itself account for the anti malarial activity whereas metabolites of primaquine may be more active than the parent compound in causing hemolysis (2).

Absorption, Fate and Excretion (24)

The absorption of primaquine is nearly complete after oral administration. After a single dose the plasma concentration reaches a maximum within three hours and then falls with an apparent elimination half time of 6 hours. The apparent volume of distribution of primaquine is several times that of total body water. Primaquine is rapidly metabolized; only a small fraction of an administered dose is excreted as parent drug. The three oxidative metabolites of primaquine identified to date are 8-(3-carboxyl-1 methyl-propyl-amino)-6-methoxyquinoline, 5-hydroxy primaquine and 5-hydroxy-6-desmethylprimaquine. The three metabolites of primaquine appear to have appreciably less anti-malarial activity than does primaquine. However except for the carboxyl derivative their hemolytic activity as assessed by formation of methemoglobin in vitro is greater than that of the parent compound (24).

Toxicity and side effects

In the usual therapeutic doses, primaquine is fairly innocuous when given to Caucasians. Mild to moderate abdominal cramps and occasional epigastric distress occur in some individuals given the larger doses, and mild anemia, synosis (methemoglobinemia) and leukocytosis have been observed (25). Higher doses (60 to 240 mg of primaquine daily) accentuate the abdominal symptoms and cause methemoglobinemia and cyanosis in most of the subjects and leukopenia in some. Hepatic function is unaffected; taking the drug at mealtime can alleviate abdominal distress (2).

Primaquine is known to inhibit SOD and catalase (26). Hydrogen peroxide is the potential toxic product formed from the primaquine metabolites (27). It is reported that primaquine was not effective on erythrocyte membrane ATPase but sera containing primaquine metabolites can inhibite various ATPases in G6PDH deficient patients (28). Na+ K+, ATPase from toad bladder is also inhibited by primaquine (29). Yeast mitochondrial functions are also affected with primaquine (30).

Quinine

History

Quinine is the chief alkaloid of cinchona, the bark of the cinchona tree indigenous to certain region of South America. The bark is also called Peruvian. Jesuit's or Cardinal's bark. The first written record of the use of cinchona occurs in a religious book written in 1633 and published in Spain in 1639. The author an Augustine monk named Calancha of

Lima, Peru wrote, "A tree grows which they call 'the fever tree'". The term cinchona was chosen by Line who accidentally misspelled it for the species of trees yielding the bark. Although this term is probably derived from the name of the countess whose alleged cure led to its wide use, some believed that it comes from world of Incan origin 'Kinia' which means bark (2.3)

Anti-malarial action

Quinine acts primarily as a blood schizontocide. It has little effect on sporozoites of pre-erythrocytic forms of malarial parasites. The alkaloid is also gametocidal for *Plasmodium vivax* and *Plasmodium malariae*, but not for *Plasmodium falciparum*. As both are suppressive and therapeutic agent, quinine is more toxic and less effective than chloroquine possibly because its activity stems primarily from its properties as a weak base. However, quinine is especially valuable for treatment of severe illness due to chloroquine resistance and multi-drug resistant strains of *Plasmodium falciparum* Mode of action is supposed to be similar to chloroquine (2,31).

Absorption, Fate and Excretion (14)

Quinine and its congeners are readily absorbed when given orally or intramuscularly. In the former case, absorption occurs mainly from the upper small intestine and is over 80% complete even in patients with marked diarrhea. Peak plasma concentrations of cinchona alkaloids occur within 1-3 hrs after a single oral dose. After termination of quinine therapy, the plasma concentration falls with a half time of about 11 hrs. Plasma concentration of quinine between 8-15 mg/lit are effective clinically and generally non-toxic. The cinchona alkaloids are extensively metabolized especially by the CYP3A4 in the liver so that only about 10% of an administered dose is excreted unaltered in the urine (32). There is no accumulation of drug in the body upon continued administration. Metabolites are excreted in the urine. Renal excretion of quinine is twice as rapid when the urine is acidic as when it is alkaline (14).

Toxicity and side effects

Fatal oral dose of quinine for adult is approximately 2-8 gm (2,3). When quinine is repeatedly given in full therapeutic doses, typical dose related clusters of symptoms occur, termed cinchonism. In mild form, this consists of ringing in the ear, headache, nausea and disturbed vision (33). However, when medication is continued after large single doses, gastrointestinal, cardio-vascular and dermal manifestation may appear. Hearing and vision are particularly disturbed Functional important of eighth nerve results in tinnitus, decreased auditory acuity and vertigo. Quinine when given intravenous can cause 33% mortality (3). Quinine is a non-specific inhibitor of Ca^{2+} activated ionic channels and is the only known inhibitor of Ca^{2+} induced vesiculation (34). Quinine specifically inhibits the proteolipid subunit of FoF1 ATPase in *S. pneumoniae* (35).the production of reactive nitrogen species is also inhibited by quinine (36)

Other available drugs

Doxycycline Hyclate

Doxycycline a widely used antibiotic is indicated for the prophylaxis of malaria due to *P. falciparum*: it is less effective against *P. vivax* infections. It is effective against asexual, erythrocytic forms of *P. falciparum*, but not gametocytes of the sexual stage. It is also indicated for treatment of resistant strains of *falciparum* malaria.

Most frequently observed side effects include nausea and epigastric distress; less frequent are incidents of diarrhea and vomiting. Stomach and esophageal ulceration has been reported. Absorption of this drug is impaired by antacids containing aluminum, calcium, magnesium, iron, or bismuth subsalicylate. Monilial vaginitis and increased sensitivity to sun exposure are also common side effects (37).

Halofantrine (Halfan7)

Halofantrine is a phenanthrenemethanol discovered and developed by Walter Reed Army Institute of Research, and subsequently codeveloped by SmithKline Beecham.

Halofantrine is effective against chloroquine-sensitive and chloroquine-resistant *P. falciparum*. It is also effective against *P vivax* and some multi-resistant strains of *P. falciparum*

Halofantrine is generally well tolerated. It may cause gastro-intestinal symptoms, including diarrhea In doses higher than normal or when taken with food containing fat it can cause prolongation of QT interval. Prior treatment with mefloquine increases the likelihood of QT interval prolongation and can also lead to torsade-de-pointes in individuals with congenital prolonged QT syndrome (2).

Mefloquine HCl (Lariam R)

Mefloquine HCl provides improved prophylaxis against chloroquine-resistant strains of *P. falciparum* and *P. vivax* (38). However, *P. falciparum* strains resistant to mefloquine have been reported (39) The most frequently observed side effect is vomiting, (3% incidence) It has also been associated with the occurrence of neurologic and psychiatric events after both prophylactic and therapeutic use. Minor neurologic events include dizziness, vertigo, headache, decrease in sleep, visual, and auditory disturbances. Serious adverse events such as seizures, disorientation, and toxic encephalopathy have also been reported (2)

Quinidine Gluconate

Quinidine is a cinchona alkaloid, the dextrostereoisomer of quinine and was used to treat cardiac arrhythmia. Now it is the drug of choice for intravenous treatment of chloroquine-resistant falciparum malaria as intravenous quinine is more toxic (14). This drug is very effective and safe for intravenous treatment of severe malaria.

Quinidine is toxic to the heart if given too quickly or in too high a dose. EKG changes including prolonged QT intervals are common, but life threatening arrhythmias are rare if proper dosages are used. Most side effects are gastrointestinal in nature and include nausea, vomiting, abdominal pain, diarrhea, and rarely, esophagitis. Symptoms of mild to moderate cinchonism (ringing in the ears, headache, nausea, and impaired vision) may appear in sensitive patients after one dose of the drug (33) Less frequent side effects include urticaria, skin flushing with intense itching, and hypersensitivity reactions of angioedema, acute asthmatic episode, and liver toxicity (2).

Atovaquone

Atovaquone is an antiprotozoal agent and is a synthetic derivative of hydroxynaphthoquinone, and may exert its effect by selectively inhibiting electron transport in mitochondria (40). Recent trials have shown that a 3 day course of 1000 mg of atovaquone and 400 mg of proguanil had a cure rate of 87% for chloroquine-resistant *falciparum* malaria (41,42).

Atovaquone is well tolerated. Common side effects listed in order of occurrence are rash, nausea, diarrhea, headache, fever, and vomiting (41,42).

Hydroxychloroquine Sulfate (Plaquenil R)

It is also a 4-aminoquinoline compound. Hydroxychloroquine sulfate has the same actions, effectiveness, and indications as chloroquine phosphate. Like chloroquine, it is a blood schizonticide active against *P. vivax*, *P. malariae* and *P. ovale*, but with limited activity against most *P. falciparum* infections (43). It does not prevent relapse in patients with *P. vivax* and *P. ovale* infections, and must be followed with primaquine to effect radical cure of these diseases.

Side effects reported include mild and transient headache, dizziness, and gastrointestinal complaints of diarrhea, loss of appetite, nausea, abdominal cramps, and rarely, vomiting (2).

Pyrimethamine/Sulfadoxine (Fansidar R)

This is a combination drug containing the DNA synthesis inhibitors pyrimethamine and sulfadoxine. Each blocks a different enzyme in the synthesis of DNA from guanosine triphosphate (44,45).

Fansidar R is useful as an alternative treatment of chloroquine-resistant *falciparum* malaria It is also often used to treat suspected malaria cases in areas where persons developing malaria symptoms cannot obtain prompt medical evaluation (46).

Fatalities have occurred due to severe reactions, including Stevens-Johnson syndrome and toxic epidermal necrosis in persons using Fansidar R as a prophylaxis. No fatal reactions have been reported when it has been used for treatment (3 tablets in a single dose). Adverse reactions, include urticaria, serum sickness, itching, conjunctival or scleral injection, nausea, vomiting, headache, and drug fever (2).

Artemisinin

Artemisinin is a sesquiterpene lactone derived from the Chinese wormwood plant *Artemesia annua* Artemisinin has long been used to treat febrile illnesses in China. There it is known as "qinghaosu" (2)

Artemisinin compounds are blood schizonticides effective against parasites resistant to chloroquine and quinine. In a trial in Thailand, artensuate tablets (100 mg initial dose, followed by 50 mg q 12 hrs for 5 days) combined with mefloquine (750 mg initial dose followed by 500 mg after 6 hrs), proved effective in curing adults with uncomplicated falciparum malaria and were more effective than artensuate or mefloquine given alone (47) No severe adverse effects have been reported in clinical trials of over 4,000 patients. Mild adverse effects include transient first-degree heart block, mild decreases in reticulocyte and neutrophil counts, elevated liver transaminases, abdominal pain, diarrhea, and drug fever (48).

Proguanil (Paludrine)

Proguanil is an antifolate agent, and was the first agent found to inhibit dihydrofolate reductase in *Plasmodia*. It was also recently released as part of the combination drug Malarone R (atovaquone and proguanil) (41,42,50).

It is useful as a prophylactic agent against *P. falciparum* and *P. vivax*. It acts too slowly to be used alone for treatment of acute malaria, but has been used successfully as part of multi-drug regimens for treatment of uncomplicated malaria.

Proguanil is safe at daily dosage levels. Side effects of nausea, vomiting, abdominal pain, and diarrhea have been experienced at higher dosages.

WR 238605 (Tafenoquine)

This is a new 8-aminoquinoline developed by Walter Reed Army Institute of Research now undergoing clinical trials and is similar in structure to primaquine, in initial tests it appears to be 10 times more active than primaquine (51). It is a tissue schizonticide and has shown some blood schizonticide activity. It is being developed as a less toxic alternative to primaquine to be used for radical cure of *P. vivax* and *P. ovale* malaria. It is under consideration for potential use for malaria prophylaxis.

The antimalarials act by inhibiting the growth of *Plasmodia* in the RBC and/or the liver cells and except the dihydrofolate reductase inhibitors do not have any specific mode of action. Majority of the antimalarials are weak bases and accumulates in the liver cells and RBCs and thus destroys the *Plasmodium* (11,31) Although it is reported for chloroquine that it accumulates in higher concentration in the parasitized erythrocytes than in non-parasitized erythrocytes, but still it accumulates in non-parasitized erythrocytes. There is every chance that the antimalarials can accumulate in tissues and effect their metabolic functions Also, these drugs are being used at higher concentration for treatment of other diseases Also these drugs are given normally to travelers or individual living in the endemic areas and thus could have adverse side effects. Toxicity and adverse effects of majority of the antimalarial drugs are known (described above), but the underlying biochemical effects have not been elucited. Hence in the present studies attempts were made to delineate the effects of the three antimalarials: chloroquine, primaquine and quinine on the mammalian systems. Studies were carried out on both rat liver as an experimental model as well as human erythrocytes and plasma. In the rat liver the effects of antimalarials treatment on lysosomal and mitochondrial membrane functions were examined while with human RBC and plasma effects on cholinesterases were evaluated. Given below is the brief account of lysosomes and mitochondria and the cholinesterases.

Lysosomes

The subcellular organelles lysosome contain about 70 different types of hydrolase's with variable substrate specificities (52). Lysosomal membrane contains 20 – 35 % of total lysosomal protein (53). Presence of cholesterol, sphingomyelin and N-acetylneuraminic acid is typical of lysosomal membranes (52,53).

Cogent evidence has now accumulated that implicates lysosomal function in the reception, transduction and progression of wide variety of effectors in diverse cell types. The ground for this proposal is inherent in the characteristic of lysosomes, a most heterogeneous and pluripotent class of organelles (54). More extensive considerations of lysosomal participation in the normal metabolic economy as well as under pathological condition, is available (52-54). Lysosomes constitute a major component of the vacuoles, a complex system of intracellular vesicles and structures wherein it was proposed that anabolic and catabolic cell functions are segregated (55). Extracellular material taken in course of invagination of plasma membranes result in the generation of phagosomes or endocytotic vesicles in the cell interior. The latter vesicles then generally fuse with a given fraction of the primary lysosomal population, yielding secondary lysosomes, which are considered sites of catabolic activity (55).

There are three distinct modes of entry into the lysosomes. The main route is by endocytosis, which depends on enclosure within vacuoles derived from invagination of the plasma membrane. In most cases this vacuole subsequently fuses with lysosomes either primary or secondary and thus becomes part of lysosomal system. The materials that can be taken up in this manner include a variety of small molecules.

A special and particularly interesting form of endocytosis is piggyback endocytosis. This term was first used by Sbarra et.al. (56) to described the non-selective uptake of solutes accompanying the uptake of substances that enter lysosomes as part of an endocytozable complex which are then released in free form as a result of processing of this complex within the lysosomes This mechanism operates in number of physiological as well as pathological situations

A number of substances are known that accumulate selectively within lysosomes sometimes at a very rapid and enormous amount; in spite of freely permeable character. A trapping mechanism must account for their selectively intralysosomal accumulation (54).

Lysosomotropic agents

The term "lysosomotropic" is used in study to designate all substances that are taken up selectively into lysosomes, irrespective of their clinical nature or mechanism of uptake (54). There are three distinct modes by which these lysosomotropic compound can enter into the lysosomes, lysosomes are involved in many pathological situations. Our growing understanding of this role has brought to light the need in the therapeutics for lysosomotropic agents endowed with certain well-defined properties. Such consideration may provide useful guidelines for development testing or screening (54).

Perhaps the most interesting aspect of lysosomotropism is that it can be conferred artificially on almost any substance, by suitable coupling with an appropriate carrier. This device can serve as a means not only of acting selectively in or on lysosomes, but also of directing drugs preferentially into a chosen cell type. Enough such examples are already known to indicate that this field of search offers rich possibilities for a novel kind of drug design with application to many different diseases including among others microbial infection, parasitosis, leukemia and cancer (52).

Mode of action of lysosomotropic compounds

The agent can remain in the lysosomes and act by altering the intralysosomal *milieu* or it can do this by sheer bulk causing the lysosomes to swell to accommodate the incoming material together with water and this in turn results in the lysosomal storage syndrome. In addition to storage, many specific effects can be exerted inside the lysosome e.g. activation of enzyme. inhibition of enzymes, change in local pH, change in ionic composition etc.

In addition to lysosomal effect, they can also show extra lysosomal effects by producing permeant substances that arises from these agents. These compounds may also show membrane effects such as stabilizing the membrane or destabilizing the membrane.
In this context studies on effects of antimalarials on lysosomal functions are desirable.

Lysosomes and Antimalarials

As is already described the antimalarial drugs are basic compounds (Chloroquine, quinine, primaquine) and hence they have tendency to migrate towards the acidic organelle inside the cell. The plasmodial vacuoles and host tissue lysosomes have acidic pH inside and and thus the antimalarials can accumulate in them. Chloroquine is a lysosomotropic agent (54) and is reported to accumulate in very high concentrations in liver lysosomes (54) Several effect of chloroquine as lysosomotropic compound are also known such as it inhibits the proteases such as cathepsins (57). Chloroquine is used to block the lysosome-dependent cellular process in the experimental studies such as activation of insulin receptors (58). It alters the membrane permeability in pancreatic lysosomes (59). It also increased the liver phospholipid content by as much as 50% and excess phospholipid was localized in lysosomes (60). In plasmodial food vacuoles it alters the activity of aspartic proteases and heme polymerases (12,13,61)..

Mitochondria

Cellular respiration is the process of oxidizing food molecules, like glucose, to carbon dioxide and water The energy released is trapped in the form of ATP for use by all the energy-consuming activities of the cell. The process occurs in two phases:-glycolysis, the breakdown of glucose to pyruvic acid and the complete oxidation of pyruvic acid to carbon dioxide and water. In eukaryotes, glycolysis occurs in the cytosol. The remaining processes take place in mitochondria.

Mitochondria are the membrane-enclosed organelles distributed through the cytosol of most eukaryotic cells Their main function is the conversion of the potential energy of food molecules into ATP.

Mitochondria have an outer membrane that encloses the entire structure, an inner membrane that encloses a fluid-filled matrix, between the two is the intermembrane space, the inner membrane is elaborately folded with shelflike cristae projecting into the matrix and a small number (some 5-10) circular molecules of DNA

The Outer Membrane

The outer membrane contains many complexes of integral membrane proteins that form channels through which a variety of molecules and ions move in and out of the mitochondrion.

The Inner Membrane

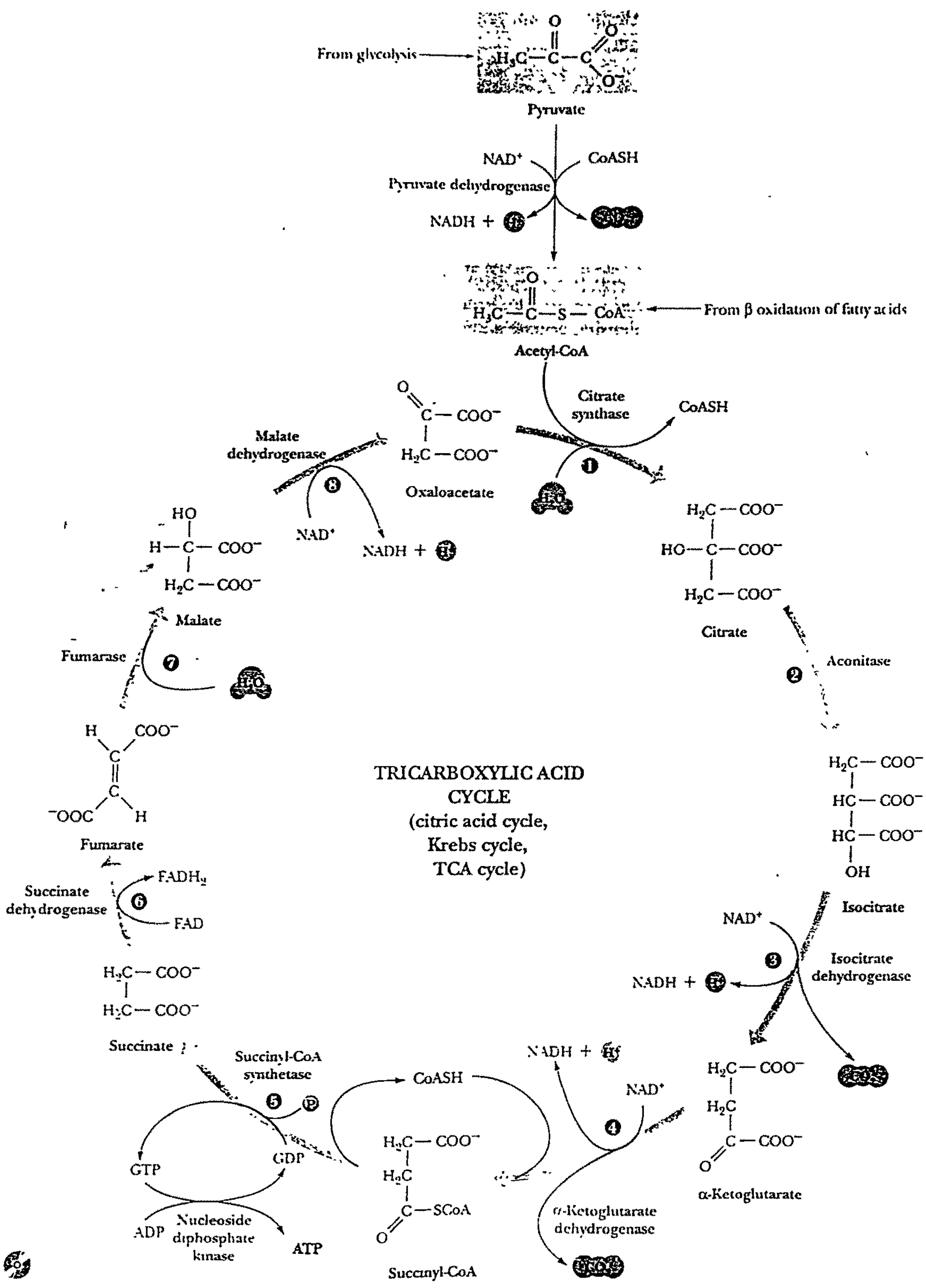
The inner membrane contains 5 complexes of integral membrane proteins·

- NADH dehydrogenase
- succinate dehydrogenase
- cytochrome c reductase(also known as the cytochrome b-c1 complex)
- cytochrome c oxidase
- ATP synthase

The Matrix

The matrix contains a complex mixture of soluble enzymes that catalyze the oxidation of pyruvic acid (citric acid cycle) and other small organic molecules.

In the citric acid cycle a pyruvic acid molecule is decarboxylated resulting in a 2-carbon fragment of acetate. This acetate is coupled to a molecule of oxaloacetic acid resulting in the formation of citric acid which undergoes the series of enzymatic steps shown in the Fig 3 (62) The final step regenerates a molecule of oxaloacetic acid and the cycle is ready to turn again. At four steps in the cycle, a pair of electrons (2e-) is removed and transferred to NAD^+ reducing it to $NADH + H^+$ and at one step, a pair of electrons is


From glycolysis
Pyruvate
NAD⁺
CoASH
Pyruvate dehydrogenase
NADH + H⁺
CO₂
Acetyl-CoA
From β oxidation of fatty acids
Citrate synthase
CoASH
H₂O
Oxaloacetate
Malate dehydrogenase
NAD⁺
NADH + H⁺
Malate
Fumarase
H₂O
Fumarate
Succinate dehydrogenase
FADH₂
FAD
Succinate
Succinyl-CoA synthetase
Ⓟ
GTP
GDP
ADP
ATP
Nucleoside diphosphate kinase
CoASH
Succinyl-CoA
NADH + H⁺
NAD⁺
α-Ketoglutarate dehydrogenase
CO₂
α-Ketoglutarate
Isocitrate dehydrogenase
NAD⁺
NADH + H⁺
CO₂
Isocitrate
Aconitase
Citrate
TRICARBOXYLIC ACID CYCLE
(citric acid cycle,
Krebs cycle,
TCA cycle)

removed from succinic acid and reduces FAD to $FADH_2$ These electrons of NADH and $FADH_2$ are transferred to the respiratory chain.

The Respiratory Chain

The respiratory chain consists of 3 complexes of integral membrane proteins

- the NADH dehydrogenase complex
- the cytochrome c reductase complex
- the cytochrome c oxidase complex

and two freely-diffusible molecules

- ubiquinone
- cytochrome c

that shuttle electrons from one complex to the next.

The stepwise transfer of electrons takes place from NADH (and $FADH_2$) to oxygen molecules to form (with the aid of protons) water molecules (H_2O). The energy released as electrons pass down the gradient from NADH to oxygen, is harnessed by the three enzyme complexes of the respiratory chain to pump protons (H^+) against their concentration gradient from the matrix of the mitochondrion into the intermembrane space. Approximately 20 protons are pumped into the intermembrane space as the 4 electrons (needed to reduce oxygen to water) pass through the respiratory chain. As their concentration increases a strong diffusion gradient is set up. The protons can flow back down this gradient, reentering the matrix, only through another complex of integral proteins in the inner membrane, the **ATP synthase** complex. As in chloroplasts, the energy released as these electrons flow down their gradient is harnessed for the synthesis of ATP. The process is called chemiosmosis.

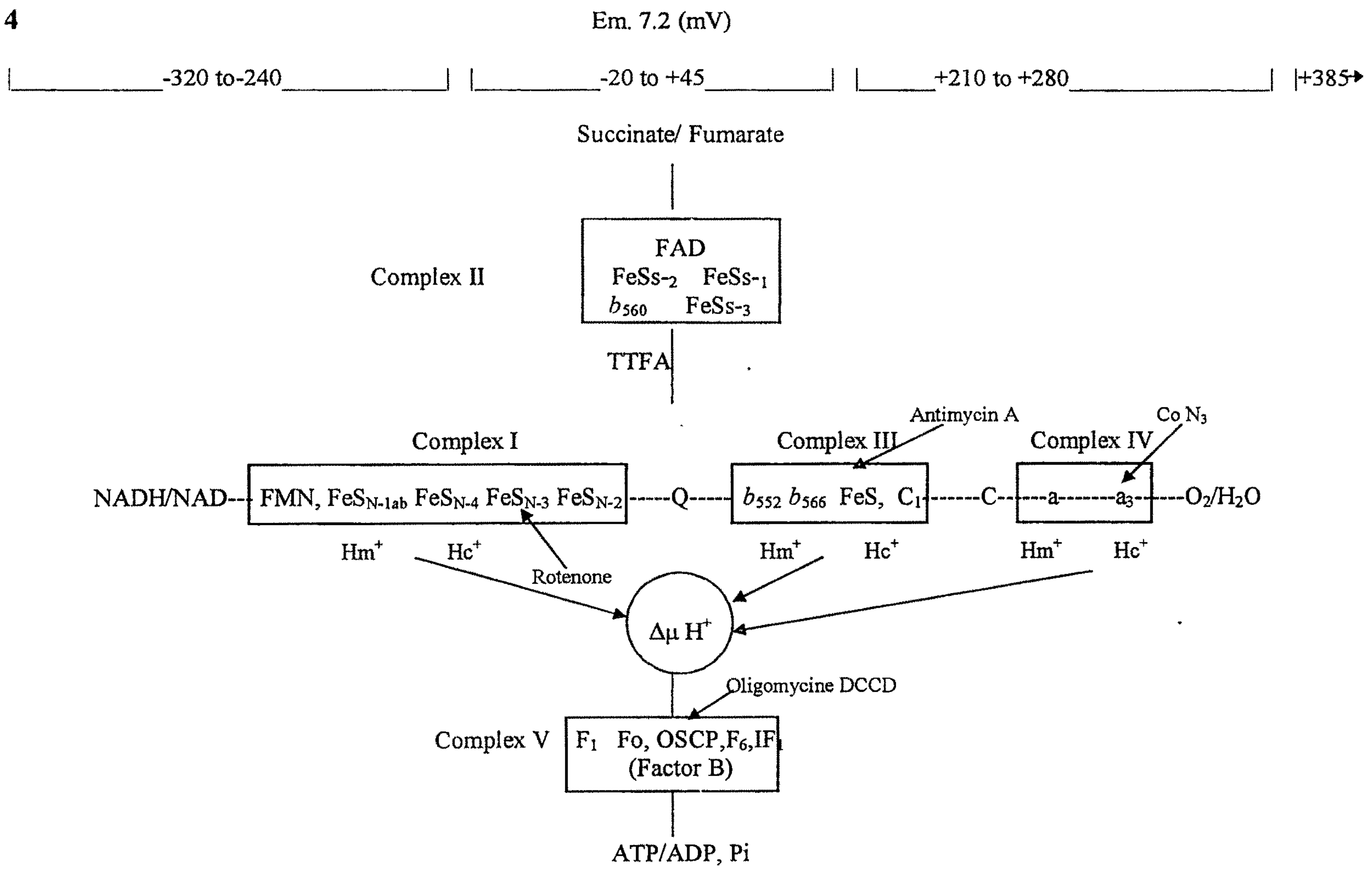
The electron transport chain and the process of ATP synthesis is shown schematically in Fig 4 (63).

Mitochondrial DNA (mtDNA)

The human mitochondrion contains 5-10 identical, circular molecules of DNA ( ). Each consists of 16,569 base pairs carrying the information for 37 genes which encode 2 different molecules of ribosomal RNA (rRNA), 22 different molecules of transfer RNA (tRNA) (at least one for each amino acid) and 13 polypeptides. The rRNA and tRNA molecules are used in the machinery that synthesizes the 13 polypeptides. The 13 polypeptides are subunits of the protein complexes in the inner mitochondrial membrane, including subunits of NADH dehydrogenase, cytochrome c oxidase, and ATP synthase. However, each of these protein complexes also requires subunits that are encoded by nuclear genes, synthesized in the cytosol, and imported from the cytosol into the mitochondrion (64).

A number of human diseases are caused by mutations in genes in our mitochondria. the defect ismostly in cytochrome b, 12S rRNA , ATP synthase, subunits of NADH dehydrogenase or several tRNA genes. Although many different organs may be affected, disorders of the brain and muscles are the most common (65).

Fig. 4



Em. 7.2 (mV)
-320 to-240
-20 to +45
+210 to +280
+385
Succinate/ Fumarate
Complex II
FAD
FeSs-2 FeSs-1
b560 FeSs-3
TTFA
Complex I
NADH/NAD
FMN, FeSN-1ab FeSN-4 FeSN-3 FeSN-2
Q
Antimycin A
Complex III
b552 b566 FeS, C1
C
Co N3
Complex IV
a
a3
O2/H2O
Hm+
Hc+
Rotenone
Hm+
Hc+
Hm+
Hc+
Δμ H+
Oligomycine DCCD
Complex V
F1 Fo, OSCP,F6,IF1
(Factor B)
ATP/ADP, Pi

Mitochondria and antimalarials

Chloroquine is known to modulate the activity of many non-lysosomal enzymes. Reports in the literature suggest that chloroquine and primaquine affect the structure and function of the mitochondria in *Plasmodium* as well as in tissue like heart (66). In a recent report Ross et al. have shown that chloroquine treatment resulted in the induction of cardiolipin synthesis (67). Quinine specifically inhibited the proteolipid subunit of FoF1 ATPase in *S. pneumoneae* (35). Deepalakshmi et al. showed that oral feeding of chloroquine resulted in increase in the total phospholipids and decrease in cholesterol in rat liver mitochondria (20). Chloroquine inhibits the activity of NADH dehydrogenase, succinate dehydrogenase, and cytochrome c oxidase (20). It is also reported that chloroquine is a potent inhibitor of glutamate dehydrogenase in liver and kidney cortex of rabbit and that chloroquine does by abstracting the electrons from the dehydrogenases (19).

Cholinesterases

For acetylcholine to serve as a neurotransmitter in the motor system and certain neuronal synapses, it must be removed or inactivated within the time limits imposed by the response characteristics of the synapse. At the neuro-muscular junction immediate removal is required to prevent lateral diffusion and sequential activation of receptors. Modern biophysical techniques have revealed that the time required for hydrolysis of acetylcholine is less than a millisecond at the neuromuscular junction (68). This function is performed by one of the fastest enzyme known i e cholinesterases (69). Cholinesterases are defined by the fact that they hydrolyze choline ester faster than other substrate and are inhibited by the natural carbamate alkaloid, physostigmine (also called eserine) (70).

Vertebrates, in particular possess two cholinesterases, corresponding to two distinct genes: acetylcholinesterase (AChE EC 3.1.1.7) and butyrylcholinesterase (BuChE EC.3 1 1.8) AChE and BuChE typically are distinguished by the relative rates of acetylcholine and butyrylcholine hydrolysis and by the effect of inhibitors (70).

The distribution of AChE and BuChE transcripts was analyzed by Northern blots in human tissues. AChE mRNA is most abundant in skeletal muscle and brain. These same tissues have high cholinesterase enzyme activity, consistent with acetylcholinesterase function in nerve impulse transmission. In contrast, the level of BuChE is very high in liver, high in lungs, low in brain and heart, barely detectable in muscle, kidney and pancreas and apparently absent in placenta (71). A high level of BuChE mRNA in liver supports the finding that liver is the source of blood plasma butyrylcholinesterase (71).

Whereas the role of AChE in cholinergic transmission is unambiguous, the function of BuChE remains a puzzle, however, it is known to hydrolyze drugs such as aspirin, succinylcholine. heroin and cocaine (72-74). In addition BuChE has a positive effect against organophosphate poison when administered as a prophylactic treatment in mice, rats and non human primates (75-78). This suggests that it may serve as a scavenging enzyme in the detoxification of natural compounds. Recently a mutant BuChE the G117H mutant has been produced which can hydrolyze organophosphates (79,80)

AChE exists as a family of molecular forms that may be classified as amphiphilic and non-amphiphilic according to their hydrophobic interactions and as homomeric and heteromeric according to their quaternary structure (81). In vertebrates these molecules are generated from a single gene· the catalytic domain may be associated with several types of C- terminal peptides that define distinct types of catalytic subunits (AChE$_S$, AChE$_H$. AChE$_T$) and determine their post-translational maturation. AChE$_S$ generates soluble monomers in the venom of Elapid snakes. AChE$_H$ generates GPI – anchored dimers in *Torpedo* muscles and on mammalian blood cells. AChE$_T$ is the only type of catalytic subunit that exists in all vertebrate cholinesterases; it produces the major forms in adult brain and muscle. Heteromeric membrane bound and collagen – tailed molecules result from the association of tetramers of AChE$_T$ subunits with an hydrophobic anchoring subunit (P) or with a triple helical collagen tail which helps them to attach them to the basal lamina or to cell membranes (81). In the collagen–tailed forms or asymmetric forms (A$_4$, A$_8$ and A$_{12}$) AChE$_T$ subunits are associated with a specific collagen, Col Q, which is encoded by a single gene in mammals. ColQ contains a short peptidic motif, the proline – rich attachment domain (PRAD) that triggers the formation of AChE$_T$ tetramers form monomers and dimers Although the COLQ gene produces multiple transcripts it does not generate the hydrophobic tail, P, which anchors AChE in mammalian brain membranes. The coordinate expression of AChE$_T$ subunits and anchoring proteins determines the pattern of molecular forms and therefore the localization and the functionality of the enzyme (81).

The sequences of several cholinesterases are available. The primary structure of the mature catalytic subunit consists of a major domain of 535 amino acids (70), The catalytic subunits of AChE are glycoproteins of 70 – 110 kDa, depending on the species.

This is followed by variable C-terminal peptides, as discussed above. The common domain is highly homologous in all cholinesterases, from invertebrates and vertebrates (82)

The active site of AChE is classically considered as being composed of two subsites, the esteratic subsite containing the active site serine and the anionic subsite which binds the quaternary ammonium residue of acetylcholine. Binding at this anionic subsite seems to involve hydrophobic as well as electrostatic interactions, as shown by comparative kinetic studies employing isosteric charged and uncharged substrates and inhibitors (83). In addition, AChE binds certain inhibitors at a distinct site, called the peripheral site (84,85). The peripheral site is thought to be involved in the phenomenon of excess substrate inhibition (86,87)

The active site serine (S200) is located near the bottom of a deep, narrow gorge, which reaches halfway through the potein. This serine seems to form a catalytic triad with H440 and with E327 The walls of the active site gorge are lined by 14 aromatic residues, including W84 and Y 330, which had been previously assigned to the active site by affinity or photoaffinity labeling (88).

The anionic site appears misnamed; the binding of the quaternary ammonium group of choline probably occurs through interactions with the π electron of aromatic groups (89).

The peripheral site, according to the labeling of peptides 251 – 264 and 270-278 (90), appears to be located on the surface of the protein, near the entrance of the active site gorge Aromatic amino acids, in particular W (279), are part of the common core of the peripheral anionic site (91) The amino acids proposed for the AChE relay pathway are W (279), Y (121), Y (70), D (72), Y(334) and Y(330) (92)

The presence of the inhibitors at the peripheral site would block access of charged substrates, but not of uncharged substrates, to the active site. Acetylcholine may therefore bind first at the peripheral site, which contains a group of negative charges ('landing site) and then slide down the aromatic lined gorge into the active site (92). The peripheral site thus has been viewed by different authors as an obligatory landing site for charged ligands (93), a part of the active site (94), an ionic strength sensor (95) and the site of excess substrate inhibition (87).

One of the proposed functions of the peripheral anionic site is to transmit a signal from the top of the gorge to the bottom of the gorge resulting in a conformational change (91,96,97) In AChE several amino acids participate in peripheral anionic site, but only two amino acids, W(279) and D (72), constitute the common core of the peripheral anionic site (91) The other amino acids in AChE peripheral site, Y (70), Y (121), E(278) and Y(334) may or may not participate in binding depending on the structure of the ligand (91)

The aromatic residues in the AChE peripheral site have been fundamental to the elegant binding studies (98 – 100) that have defined the characteristics of the peripheral anionic site in *Torpedo* AChE. These binding studies showed that *Torpedo* AChE undergoes a conformational change following binding of peripheral–site ligands; they provided evidence that different arrays of amino acids are involved depending on the identity of the ligand

Human butyrylcholinesterase (EC 3 1.1.8; BuChE) is a 340 kDa tetrameric sialoglycorotein organized as a dimer of dimers. There is one active site per subunit of 85 kDa. Subunits in the dimer are held together by a disulphide bridge at the subunit C terminal (101) The whole polypeptide of human BuChE contains the same number of amino acids as that of *Torpedo* AChE, with a large proportion of conserved residues. It was, therefore, possible to obtain a three-dimensional model of BuChE by replacing all the different residues in the structure of AChE (102). The resulting model of BuChE contains an identical catalytic triad, but differs from AChE by the smaller number of aromatic amino acids in the walls of catalytic gorge (8 instead of 14). As for AChE, the active site serine [S192(200)] of BuChE is located at the bottom of a narrow (4A) and deep (20A) gorge (89,102). In human BuChE, residues D70 (103,104) and Y332 (105) have been found to be components of the peripheral anionic site (PAS). Human BuChE has previously been thought not to have a peripheral anionic site (106). The reason for this lies in the definition of the peripheral anionic site, a definition which is still undergoing modification. In BuChE, communication between D70 in the peripheral site and W82 in the choline binding site seems to require nothing more than the presence of a positively charged ligand. It has been proposed that the role of the negatively charged D70 is to orient the positive charge of the substrate onto W82. Aspartate 70 [D70] at the rim of the gorge is the initial substrate-binding site of positively charged substrates (92). After the formation of the initial encounter complex, the substrate molecule is thought to slide down the gorge to reach tryptophan 82 [W 82(84)], the actual 'anionic' site, to form the productive Michaelian complex (92). The D70residue (D72 in *Torpedo* AChE) as a key element of PAS is also important for control of substrate activation, inhibition and aging of cholinesterases (103,104,106).

The pronounced substrate activation of wild type BuChE by butyrylthiocholine (107) is in contrast to the substrate inhibition observed for AChE with acetylcholine (92,107), but both have been interpreted as binding an additional substrate molecule to a peripheral regulatory site (107). In BuChE the second binding site could be located inside the active–site gorge, though transient binding to D70 at the entrance of the gorge may take place

AChE residues that have been identified as affecting substrate inhibition are D(72), W(279), and Y(334) (92) in the peripheral site, as well as residues F(290), Y(330), F(331) and E(199) inside the gorge (92,107).

The inhibitors of cholinesterases can be reversible and irreversible (68). As described, three distinct domains on AChE constitute binding sites for inhibitory ligand and form the basis for specificity differences between AChE and BuChE: the acyl pocket of the active center, the choline subsite and the peripheral anionic site (68) The inhibitors broadly fall in two groups: firstly those compounds which have a quaternary ammonium group or secondly those which belong to the family of organophosphorous compounds. Inhibition with the former is considered to be reversible while the inhibition with the later group of inhibitors is of irreversible type (68). However, reactivation of the enzyme inhibited by organophosphorous compound using suitable thiol compound or derivative of hydroxamic acid, oximes and bis-quaternary oximes has been demonstrated (108).

Reversible inhibitors such as edrophonium and tacrine bind to the choline subsite in the vicinity of W 86 and H 202 whereas donepezil binds to the active center (109).

Propidium is the classic peripheral anionic-site ligand of *Torpedo* AChE (98). Propidium binds exclusively to the peripheral site of *Torpedo* AChE and not to the active site.

Fasciculin-2, a 61-amino acid peptide from the venom of the African green mamba snake, binds exclusively to the entrance of the active site gorge of AChE (110). The binding to mouce AChE is extremely tight having a Ki value of 2pM (110), whereas binding to human BuChE is six orders of magnitude weaker, being 2uM. Radic et al. identified three amino acids which accounted for the huge binding affinity difference in AChE and BuChE These amino acids are Y (70), Y (121) and W (279) in wild type mouse AChE (110)

Dibucaine has been used for the past 30 years to phenotype butyrylcholinesterase genetic variants in more than 100000 people (2,3). The dibucaine accurately predicts the presence of D70G mutation which indicates that dibucaine interacts with D70 (111,112).

Drugs that have a carbamoyl ester linkage, such as physostigmine and neostigmine attach to the active site serine and give rise to carbamoylated enzyme. The carbamoyl moiety resides in the acyl pocket outlined by F295 and F297 (68). In contrast to the acetylated enzyme, methylcarbamoyl AChE and dimethylcarbamoyl AChE are far more stable. Sequestration of the enzyme in its carbamoylated form thus precludes the enzyme catalyzed hydrolysis of ACh for extended periods of time (68).

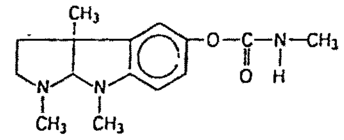
The organophosphorous inhibitor such as di-isopropyl fluorophosphate (DFP), serve as true hemi-substrate, since the resultant conjugate with the active site serine phosphorylated or phosphonylated is extrmely stable (68).

The structures of some known inhibitors of cholinesterases are given in Fig. 5-6.

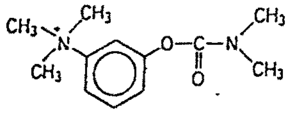
The single most studied tissue with regard to human cholinesterases is blood. Human red blood cells are a source of acetylcholinesterase enzyme uncontaminated with butyrylcholinesterase, while human plasma is a source of butyrylcholinesterase enzyme virtually free of acetylcholinesterase (113). Human serum contains 5mg BuChE per liter. Human red blood cells contain 0.5mg per liter of packed red cells. Packed red cells take upto 40% of the volume of blood, so that the concentration of AChE in whole blood is 3 nM and BuChE is 36nM. Thus, the amount of BuChE in human whole blood exceeds the amount of AChE by more than 10 fold. The AChE on human red blood cells is anchored to membrane via a C-terminal glycophospholipid (114).

Because the physiological role of AChE is absolutely vital and because vertebrates posseses a single gene for this enzyme, there are no known defects in the structural gene itself (70), although there are many anomalies in the molecular properties of the enzyme, e.g. in the proportions of its molecular forms or in their distribution (68,70). This is in marked contrast to the several types of more or less defective varients of BuChE, which appear to be largely dispensable (70). Human pathologies, which are associated with cholinesterases, include paroxysomal nocturnal hemoglobinuria (115), neural tube defects (70), Myasthenic syndromes (116) Grave's opthalmopathy (117) and Alzheimer's disease (118,119).

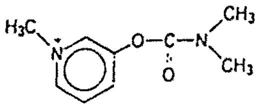
Fig. 5



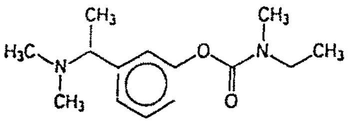
PHYSOSTIGMINE



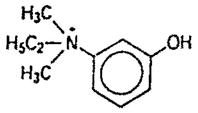
NEOSTIGMINE



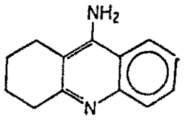
PYRIDOSTIGMINE



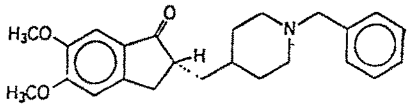
RIVASTIGMINE



EDROPHONIUM



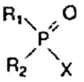
TACRINE



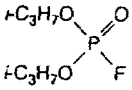
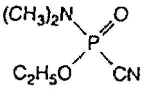
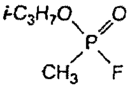
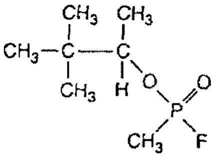
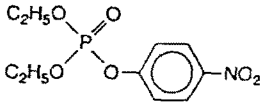
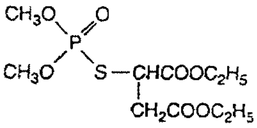
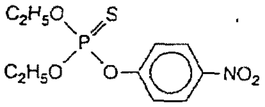
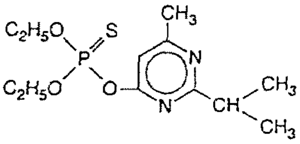
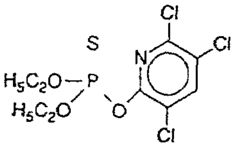
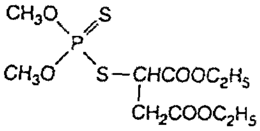
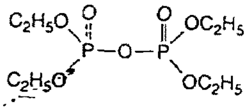
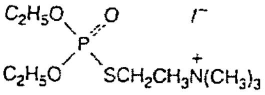
DONEPEZIL

Chemical Classification of Representative Organophosphorus Compounds of Particular Pharmacological or Toxicological Interest

General formula (Schrader, 1952):



Group A. X = halogen, cyanide, or thiocyanate leaving group, group B. X = alkylthio, arylthio, alkoxy, or aryloxy leaving group, group C, thionophosphorus or thio-thionophosphorus compounds; group D, pyrophosphates and similar compounds, group E, quaternary ammonium leaving group

GROUP	STRUCTURAL FORMULA	COMMON, CHEMICAL, AND OTHER NAMES	COMMENTS
A		DFP, Isoflurophate, diisopropyl fluorophosphate	Potent, irreversible inactivator
		Tabun Ethyl *N*-dimethylphosphoramidocyanidate	Extremely toxic "nerve gas"
		*Sarin (GB)* *Isopropyl methylphosphonofluoridate*	Extremely toxic "nerve gas"
		Soman *Pinacolyl methylphosphonofluoridate*	Extremely toxic "nerve gas"
B		Paraoxon (MINTACOL), E 600 *O,O*-Diethyl *O*-(4-nitrophenyl)-phosphate	Active metabolite of parathion
		Malaoxon *O,O*-Dimethyl *S*-(1,2-dicarboxyethyl)-phosphorothioate	Active metabolite of malathion
C		*Parathion* *O,O-Diethyl O-(4-nitrophenyl)-phosphorothioate*	*Employed as agricultural insecticide, resulting in numerous cases of accidental poisoning. Will be phased out of agricultural use by October, 2003.*
		Diazinon, Dimpylate *O,O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate*	*Insecticide in wide use for gardening and agriculture. Now banned for indoor use and being phased out of all outdoor use by 2005*
		Chlorpyrifos *O,O*-Diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate	*Insecticide with restricted use in consumer products and limited to nonresidential settings*
		Malathion *O,O*-Dimethyl *S*-(1,2-dicarbethoxy-ethyl) phosphorodithioate	Widely employed insecticide of greater safety than parathion or other agents because of rapid detoxification by higher organisms
D		TEPP Tetraethyl pyrophosphate	Early insecticide
E		Echothiophate (PHOSPHOLINE IODIDE), MI-217 Diethoxyphosphinylthiocholine iodide	Extremely potent choline derivative; employed in treatment of glaucoma, relatively stable in aqueous solution

A loss of the cholinergic innervation of the cortex seems to be primary importance in the development of Alzheimer's disease. For these reason the therapeutic potential of cholinesterase inhibitors are being actively explored (68,70). Cinchona alkaloids are reported to inhibit cholinesterases (71). A few reports also suggest that chloroquine inhibits eel AChE and horse serum BuChE and microsomal AChE (15,21). Recently anti-inflamatory compounds were also reported to have beneficial effects in Alzheimer's disease (120,121) This suggests that chloroquine and other quinoline compounds – which show anti-inflamatory properties can be useful in treatment of Alzheimer's disease and if they can show potent inhibition properties for human AChE and BuChE, they can open newer avenues for treatment of Alzheimer's disease. In the light of this studies were carried out to check the potential of the three antimalarials quinine, chloroquine and primaquine to inhibit cholinesterases. The AChE of RBCs presents the almost pure form without the contamination of BuChE awhile the plasma BuChE is practically free from AChE (113). Hence it was felt that comparative studies using these two systems can give better insights in the possible interaction of the antimalarials with these two related enzyme systems having different functions (70).

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