CHAPTER 1

INTRODUCTION

Filariasis remains a public health problem of considerable magnitude in many tropical countries. The number of cases of lymphatic filarial disease, mostly caused by \textit{Wuchereria bancrofti} in India is greater than in any other country. Over 300 million people live in zones where lymphatic filariasis is endemic. It is estimated that there are at least 6 million attacks of acute filarial disease per year and 15 million persons currently have one or more chronic filarial lesions. Although there have been several successful programmes to control lymphatic filariasis, the disease continues to be a socioeconomic and public health problem in these tropical countries.\footnote{1}

Filariasis is a group of disorders caused by infection with the thread-like nematodes of the superfamily Filarioidea. These worms invade the lymphatics and the subcutaneous and deep tissues of human beings producing reactions ranging from acute inflammation to chronic scarring. The viviparous female discharges microfilariae (mf) into the blood or subcutaneous tissues where they live for weeks or months until taken up by hematophagous arthropods. In the body of these vectors, they are
transformed into filariform larvae which then infect a new host when the arthropod takes another blood meal. The clinical picture produced by the various species in this group are more or less specific. The term lymphatic filariasis is commonly used to designate the disease produced by *Wuchereria bancrofti* and *Brugia malayi* - the organisms responsible for lymphatic blockade and elephantiasis. *Loa loa* causes loiasis, a disease characterized by transient subcutaneous swellings, and *Onchocerca volvulus* produces blindness and pruritic skin rash typical of onchocerciasis. The parasites responsible for human filariasis in India are *W. bancrofti* and *B. malayi*. Those producing bovine filariasis are *Setaria digitata* and *Setaria cervi*. The classification of filarial parasites is shown in Scheme I.

The incidence of lymphatic filariasis is greatest in China, India and Indonesia. These three countries account for about two-thirds of the total persons infected in the world. There are extensive endemic areas in many countries of the African region; but detailed epidemiological studies have been confined to a few of those in East and West Africa. The disease is a great problem in many countries with small populations, especially in the Pacific area. The distribution of the endemic areas of the lymphatic filariasis is shown in Map I. In India it is distri-
buted chiefly along the sea coast and along the banks of big rivers; it has also been reported from Rajasthan, Punjab, Uttar Pradesh and Delhi. It is perhaps the number one public health problem in Kerala. The shaded portions in Map II and III (Fig.IA and IB) are the established endemic areas in the whole of India and in Kerala state respectively.

**Wuchereria bancrofti**

**Life Cycle**

*W. bancrofti* completes its life cycle in two hosts—*man* and *mosquito*. The definitive host is man, in whose lymphatic system the adult worms are harboured. Live embryos (mf) are discharged which find their way into the blood stream. The embryos are capable of living in the peripheral blood for a considerable period without undergoing any developmental metamorphosis. They are subsequently taken up by the female Culex mosquitoes during their blood meal. The intermediate host is the mosquito, in which the mf undergo further development and they become infective to man. A large number of species of mosquitoes belonging to the genus Culex, Aedes and Anopheles act as intermediate hosts for *W. bancrofti*.

Sheathed mf ingested by the mosquito during its blood-meal localize around the anterior end of the stomach. They cast off their sheaths, penetrate the gut wall within
an hour or two and migrate to the thoracic muscles. Here they rest and begin to grow. Within the next 2 days, the slender, snake-like organism changes into a thick, short, sausage-shaped form with a short spiky tail, measuring 124 to 250 µm in length and 10 to 17 µm in breadth. This is the first-stage larva (L₁). During the next five days, the larva grows rapidly, moults once or twice and at the end of this stage measures 225 to 330 µm in length by 15 to 30 µm in breadth. This is the second-stage larva (L₂). On the 10th or 11th day the metamorphosis becomes complete. This is the third-stage larva (L₃) which measures 1500 to 2000 µm in length and 18 to 20 µm in breadth. Now the larva is infective to man and enters the proboscis sheath of the mosquito on or about the 14th day. There may be several larvae remaining coiled up, waiting for an opportunity to infect man while the mosquito is having the blood-meal. The time taken for the complete development of mf in the mosquito varies from 10 to 20 days or more, depending on the atmospheric temperature, humidity and to a certain extent, the species of the mosquito.

When the infected mosquito bites a human being, the infective larvae are deposited on the skin near the site of puncture. Later, attracted by the warmth of the skin, the larvae either enter through the puncture wound or penetrate the skin on their own.
The infective larvae having penetrated the skin, reach the lymphatic channels, settle down at some spot and begin to grow into adult forms. In course of time, probably after a period of 5 to 18 months they become sexually mature. The male measures 2.5 to 4 cm in length and 0.1 mm in thickness. The female measures 8 to 10 cm in length and 0.2 to 0.3 mm in thickness. The male fertilizes the female and the gravid females give birth to mf. A new generation of mf is emitted which pass either through the thoracic duct or the right lymphatic duct, to the venous system, pulmonary capillaries and then to the peripheral circulation, thus completing the cycle. They are very active in their habitat and can move both with and against the blood stream. When unstained, they appear as colourless and transparent bodies with blunt heads and rather pointed tails. The mf measures about 290 μm in length and 6 to 7 μm in breadth. The life cycle of W. bancrofti is illustrated in Scheme II. The mf of W. bancrofti and B. malayi are shown in Fig.I.

**Periodicity of bancroftian mf**

The mf prevalent in India and China are not constantly found in the peripheral blood, but appear periodically at night mostly between 10 p.m and 4 a.m thus showing a nocturnal periodicity. It has been suggested that during daytime they retire principally inside the capillar-
eries of lungs, kidneys, heart and the big arteries such as the carotid. The reason for this nocturnal periodicity is not yet clearly known but it is presumed to be in some way related with the night-feeding habit of its intermediate host Culex quinquefaciatus.

In the Pacific islands, bancroftian mf does not exhibit any periodicity, being found in the peripheral blood during day and night, in equal numbers. In this case, however, the intermediate host is Aedes polynesiensis which feeds during day time also.4

Clinical manifestations

Bancroftian filariasis is characterized by a wide spectrum of clinical manifestations, the signs and symptoms often differing from one endemic area to another. The disease manifestations can be divided into two distinct clinical types; one caused by juvenile or adult worms in the lymphatic system, commonly referred to as lymphatic filariasis, the other, caused by an immune hyper-responsiveness of the human host to mf, resulting in occult filariasis, which includes tropical pulmonary eosinophilia (TPE).

The clinical course of lymphatic filariasis is often asymptomatic with subsequent episodes of acute adenolymphangitis and finally the development of chronic lymphatic obstruction. In previously unexposed persons who
move from non-endemic to endemic areas, this progress is often accelerated with early acute manifestations being followed much more rapidly by the chronic signs.

Acute bancroftian filariasis may commence with malaise and fever, followed by lymphadenitis in the groin or armpit and a typical retrograde lymphangitis. The acute attacks of adenolymphangitis, etc. in bancroftian filariasis may last for 3-15 days and may occur several times a year in the same individual. In India the incidence of acute disease has been reported to be 1-2% per half year.

The chronic stage of filariasis usually develops 10-15 years after the onset of the first acute attack. The incidence and severity of chronic clinical manifestations tend to increase with age. Hydrocoele, elephantiasis, and chyluria are the main characteristic features of chronic bancroftian filariasis. In a highly endemic area in India the annual incidence of chronic filarial pathology is about 0.5%. Elephantiasis begins as lymphoedema. The legs, scrotum, arms, penis, vulva, and breasts, are usually affected in that order of decreasing frequency. In some endemic areas such as Indonesia and the Pacific area, the whole leg or arm may be affected. However, in Africa, India and Sri Lanka the swelling often remains below the knee. In the initial stage, the swelling can best be observed around the ankles, from which it gradually spreads to the back of the
foot, the leg, and the thigh. The affected limb may increase up to more than three times its original size. Elephantiasis is usually preceded by periodic attacks of adenolymphangitis, although occasionally it develops without any such previous episode. A typical elephantoid leg is shown in Plate I.

The term occult filariasis refers to filarial infections in which the classical clinical manifestations are not present and mf are not found in the blood, although they may occur in the internal organs or tissues. Occult filariasis is believed to result from a hypersensitivity reaction to filarial antigens derived from mf. The best known example is TPE; but it is possible that in endemic areas, occult or cryptic forms of filarial infection account for several other clinical entities. In India, the annual incidence of TPE was found to be 10 cases per 100000 individuals.¹

Pathology

Invasion of human body by the infective larvae of *W. bancrofti* usually occurs without causing any symptoms. Only after moulting to fourth-stage larvae and young adults, do they begin to induce local inflammatory reactions. While most of the pathology associated with infection occurs around adult worms in the lymph nodes and afferent lymphatic
vessels, some pathology may develop elsewhere, either around mf cleared from the blood or around adult worms located in ectopic sites. Such pathological changes have cell-mediated, humoral and foreign-body components, but little is known about the factors that modulate the sequence and intensity of these reactions since only very few direct observations have been made on human tissue.

As a result of the associated lymphatic obstruction, lymphoedema and elephantiasis develop progressively. Affected tissues first become oedematous and then characteristically develop proliferative dermal changes with subsequent dermal and subcutaneous fibrosis. Increased number of mast cells have also been described in these affected tissues.¹ ⁷

Immunosuppression

Patients with any form of lymphatic filariasis except tropical pulmonary eosinophilia are, in general, poorly responsive to filarial antigens.⁸ This hyporesponsiveness appears limited almost exclusively to parasite antigens and is the most prominent in patients with microfilaraemia.⁹-¹¹ Its existence is presumably important for the successful persistence of the parasite within the host and its effects extend clearly to both cellular and humoral immune mechanisms.¹²
Antigens

The antigens of greatest practical importance in filariasis are those related to immunodiagnosis, immunopathology and protective immunity. Since these are molecules that are defined functionally, their analysis should also be closely integrated with functional studies. Thus immunoochemical techniques such as antibody-affinity chromatography, immunoprecipitation etc. are useful for purifying antigens.12

The most significant advance in the study of antigens from filarial parasites of man has been the development of techniques for rearing B. malayi and B. timori intraperitoneally in laboratory animals and jirds.13 While intact parasites and crude somatic extracts continue to be useful in diagnostic and clinical studies, many recent studies have focused on the analysis of either surface or excretory-secretory antigens since these appear to have greater stage and species specificity.14, 15.

Analysis of surface and somatic antigens using biochemical, immunoochemical and radio-labelling techniques have also been carried out on a variety of other filarial parasites including S. digitata, D. vitae, O. gibsoni, L. carinii, B. pahangi and D. immitis.14, 16-21
Immunodiagnosis

Definitive diagnosis of filarial infections requires direct demonstration of the parasite in the host. However, because adult filarial worm may remain sequestered in inaccessible sites and the means to detect them parasitologically are relatively insensitive, immunodiagnostic techniques have been routinely employed for diagnosis. Traditionally, these have been serological or skin test determinations of antibodies generated by the host. All such assays share the major drawback of being unable to discriminate between past exposure and current infection and because the magnitude of the response bears no relation to the parasite burden, recent attention has instead been focused on the direct detection of parasite antigens in patient’s blood or urine, an approach that yields a ‘parasitological’ diagnosis by employing techniques with sensitivities dramatically increased by immunological means.12

Treatment

Diethylcarbamazine (DEC), given orally, has a rapid destructive effect on mf and a slower action against adult filariae. The dosage is up to 9 to 12 mg/Kg body weight daily in three divided doses for 21 days. This course may be repeated twice at intervals of four to six weeks. Tight bandaging or bed rest with suspension or raising of the
affected part, by reducing the swelling will give temporary relief.22

Prevention

In endemic areas treatment of the whole population with DEC, 100 mg for adults and 50 mg for children, three times daily for seven days has reduced infection by robbing the mosquitoes of fresh sources of mf. This mass treatment should be combined with control of the vector by insecticides. Personal protection is obtained by wearing protective clothing and the use of insect repellents. Where the vector is a night-biting mosquito wire-screening of the house or the use of mosquito nets prevents infection. Early chemotherapy prevents later elephantiasis.22

*Setaria digitata*

The animal model taken for the study is *S. digitata* the bovine filarial parasite. *S. digitata* occurs in the peritoneal cavity of cattle and buffalo. The male measures 40-50 mm and the female 60-80 mm in length. The distance between the dorsal and ventral prominences of the prebuccal ring is 65-75 μ and the mouth is round. The tail of the female ends in a simple button and the male has 2 spicules. The mf are sheathed and measure 240-260 μ. Development occurs in mosquitoes such as *Armingeres obturbang,*
Aedes togoi, Anopheles lyrcanaus and in Culex quinquen- 
faciatus. The adult S. digitata are shown in Plate II.

Biochemistry of Filarial Parasites

Due to marked environmental differences like oxygen tension, pH variation and nutritional status, the chemical composition of filarial parasites differ from species to species and also during the individual developmental stages. Rathaur et al. described the chemical composition of mf and adult stages of S. cervi, to discover whether a specific chemical pattern exists during development. The fluid contents of adult worm and mf were approximately 75 and 90% respectively and the dry matter was composed mainly of carbohydrate, lipid, protein and traces of nucleic acid.

The parasites living in oxygen deficient habitats or in environments with periodic oxygen deficiencies usually have high carbohydrate reserves for survival under adverse conditions. The carbohydrate content of S. cervi is about 3-4 times more than that of the crow filarial worm Chandlerella hawkingi. Considerable changes were observed in glycogen content during the life cycle of parasites Mf of S. cervi contained very little glycogen compared to adult worms. The glycogen content of S. cervi adult was similar to that of
D. immitis while adults of L. carinii, C. hawkingi, and D. uniformis contain small amounts of glycogen.  

Reducing sugars accounted for 67 and 52% of the total carbohydrate respectively in adult and mf of S. cervi while the corresponding value for C. hawkingi was 40%. Since glycogen accounted for most of the carbohydrate in adult S. cervi, it appears that the parasite did not possess substantial quantities of free sugars. On the other hand mf of S. cervi contained substantial amounts of reducing sugars and little glycogen.

Glucosamine and uronic acid, the components of structural polysaccharide, were present in significant amounts in both stages of S. cervi.  

Total lipids accounted for 9 and 12% of the dry weight in adult and mf of S. cervi. Phospholipids constituted the major portion of total lipids in both the stages of S. cervi. Gupta and Kalia reported the presence of free amino acids in S. cervi adults.

Nucleic acids were present only in trace amounts in adult S. cervi while significantly higher amounts were detected in the mf stage of the parasite which is a developing and more active stage of the parasite.

Anwar et al. indicated the presence of enzymes of phosphorylative glycolysis, pentose oxidative pathway and phosphoenol-pyruvate-succinate pathway in adult females of
**S. cervi.** Mf of *S. cervi* are equipped with the enzymes of glycolysis, pentose phosphate and phosphoenol-pyruvate-succinate pathway and thus resemble the adult form in its metabolic pattern. Malate dehydrogenase was the most active enzyme in mf followed by lactic dehydrogenase and fumarase, while phosphoglucoisomerase, phosphoenol pyruvate carboxykinase and fructose diphosphate aldolase were comparatively less active. Centperazine and diethylcarbamazine significantly inhibited phosphoenol pyruvate carboxykinase, fumarate reductase and succinate dehydrogenase, suggesting that these antifilarials probably exert microfilaricidal activity by blocking the phosphoenol-pyruvate-succinate pathway.\(^2^9\)

Extracts of adult *B. pahangi* and *D. immitis* were shown to contain the enzyme related to folic acid metabolism.\(^3^0,\)\(^3^1\) Jaffe et al.\(^3^2\) also detected thymidine kinase activity in homogenates of adult *D. immitis* and *B. pahangi*. Homogenates of adult *D. immitis* possess a microsomal enzyme system able to transfer mannose from GDP mannose to endogenous lipid intermediates and exogenous dolichol monophosphate.\(^3^3\)

Activities of amylase, alkaline phosphatase, ATPase, and creatine kinase were detected in *W. bancrofti* mf homogenate.\(^3^4\) *W. bancrofti* mf also contain choline esterase, which plays an important part in their neuromuscular
The presence of alkaline phosphatase close to the cell membrane suggests an association between alkaline phosphatase and the cell membrane transport. The presence of acid phosphatase in the excretory and anal vesicles of W. bancrofti mf suggests that these structures are involved in some absorptive or secretory function.

Although much progress has been made in the control of lymphatic filariasis, there are still many unresolved problems concerning the detection of the infection, treatment and control of the disease. At the present state of diagnostic capability, which depends on demonstrating mf in the blood, "the infection rate", even in the most heavily infected countries, is seldom recorded as more than 50%. Of course this does not include the large number of mf negative individuals who have clinical signs of the disease. This is consequent upon the disappearance of mf from the peripheral blood in many people with chronic manifestations of the disease. The estimate for the "number of persons infected" therefore includes both people who are mf positive and those who are clinically positive but mf negative. If sensitive biochemical and immunological techniques could be used to supplement parasitological and clinical diagnosis, the figure for the total number of people infected would probably be much higher, for it would include all those infected persons who are asymptomatic and amicrofilaraemic.
Systematic biochemical studies were carried out on clinically important enzymes (lactate dehydrogenase, malate dehydrogenase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and alkaline phosphatase) in mf positive and normal human sera to find out a diagnostic test to detect infected persons even if they are mf negative. The effect of heat on the activity of lactate dehydrogenase isoenzymes in mf positive sera showed remarkable difference when compared to normal sera. Haematological study was also carried out on mf positive blood and normal blood. Since every third person in India faces the risk of exposure to filariasis, it is interesting to study whether there is any relationship between ABO blood group system and infectivity.

The nonavailability of human filarial parasites necessitates the use of animal filarial worms for biochemical and immunological studies. The model used for work reported in this thesis is S. digitata, a cattle filarial parasite. The lactate dehydrogenase of S. digitata was purified and characterized. The antigenicity of the different worm preparations were compared using various techniques. Development of a laboratory model for S. digitata was also included in the study. The peritoneal cavity of rat was found to be a good environment for inoculating the mf of S. digitata for haematological and biochemical studies.
Scheme I

Classification of filarial parasites

Phylum : ASCHELMINTHES
Class : NEMATODA
Subclass : PHASMIDIA
Superfamily : FILARIOIDEA

Genus

Wuchereria  Brugia  Onchocerca  Dipetalonema  Manso-  Diro-  Loa  Litomo-  Seta-  Fole-  Stephano-  Elaeophora

nella  filaria  soides  ria  yella  filaria  phora
MAP-2

FIG. I A Filaria map of India (1958) (Indian Council of Medical Research, 1971).

- AREA SURVEYED: *W. bancrofti*
- AREA SURVEYED: *B. malayi*
- AREA KNOWN TO BE FILARIOUS
MAP-3 FIG. I B MAP OF KERALA

(ISHAH & HRASAD IJMR 1967)
HOST (MAN)

Infected Mosquito
Sucking Blood

Mosquito (Vector)
Sucking infected Blood (mf)

Development of Infective Larva inside Mosquito

Life Cycle of Wuchereria Bancrofti
Morphology of *W. bancrofti* mf

*W. bancrofti* mf

*P. malayi* mf
BANCROFTIAN ELEPHANTIASIS OF HUMAN LEG
SETARIA DIGITATA

MALE AND FEMALE ADULT WORMS