PN-ABB-108

AGENCY FOR INTERNATIONAL DEVELOPMENT

WASHINGTON D.C. 20523

DATE: 9/23/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI. Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 7.272

PR #2

Attachment

PROGRESS REPORT NO. 2

EARLY DETECTION OF LYMPHATIC FILARIASIS AND ATTEMPT AT DEFINING PROTECTIVE ANTIGENS

A RESEARCH PROJECT
USAID/PSTC PROGRAM
Grant No. 936-5542-G-00-7030-00

Submitted by

WANLOP CHUSATTAYANOND, PH.D.

PROJECT LEADER

DEPARTMENT OF MICROBIOLOGY

FACULTY OF SCIENCE, MAHIDOL UNIVERSITY

PROJECT PROFILE

Country:	Thailand
Grant No.:	936-5542-G-00-7030-00
Program:	Program on Science and Technology Cooperation
Project Title:	Early Detection of Lymphatic Filariasis and Attempt at Defining Protective Antigens
Project Leader:	Dr. Wanlop Chusattayanond
Organization:	Faculty of Science, Mahidol University Bangkok 10400, Thailand
Co-investigators:	Dr. Prasert Sobhon Dr. Somphong Sakaphong Dr. Chantakorn Shutidamrong Dr. Peter Billings Dr. Araya Dharmkrong-at
Project Consultants:	Dr. David A. Denham London School of Hygiene and Tropical Medicine University of London Dr. Pornchai Matangkasombut Faculty of Science, Mahidol University
Authorized Officer:	Dr. Pairote Prempree Dean, Faculty of Science Mahidol University
Total Project Budget:	US $149,930
Project Duration:	3 years
Reporting Period:	February 1988 - July 1988
Budget Allocation for This Period:	US $21,593.06

INTRODUCTION AND OBJECTIVES

Lymphatic filariasis is a serious disease effecting approximately 90 million people in the tropics with a population at risk of more than 900 millions. It is mosquito-transmitted disease which is caused by *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. While *W. bancrofti* is found throughout the wet tropical world, *B. malayi* is confined to Asia and *B. timori* is only found on the islands of Timor, Flores, Alar and Roti of Indonesia (Wheeling *et al*, 1975).

In Thailand, *W. bancrofti* is found in the Western part of the country along the border with Burma while the endemic area of *B. malayi* is in the South. It has been reported that the control programme of filariasis in Southern Thailand during 1961-1979 which covered 6 endemic areas of malayan filariasis with a total population of about 1.5 million (people at risk) was successful. However, the Filariasis Division, Ministry of Public Health recently (1982-1985) reported that new areas which had been previously inaccessible to governmental personnels were found to be highly endemic with infection rates of 1.16% to 4.6%. As is often the case epidemiological data reflects the extent and limitation of the survey carried out and usually represent underestimation of the problems. In Thailand and most developing countries, this understimation is found all the time due to under-reporting and relative limitation of presonnels and inavailability of proper diagnostic tools for field surveys. Diagnostic test which have been currently used is the detection of microfilaraemia which occur at night. Therefore, development of a simple, sensitive, specific, accurate and affordable diagnostic systems will make case detection much simpler. Furthermore, a diagnostic system which can detect antigens of early human stages of the parasite in asymptomatic subjects in endemic areas will not only make

chemotherapy more effective but will also help to interrupt the life cycle of the parasites. By treating the subjects before they become microfilaraemic, transmission of the parasites will be reduced.

The overall aim of this research is to improve disease control by developing better immunodiagnostic tests for lymphatic filariasis as well as new tests for the detection of early, i.e., premicrofilaraemic stage of filarial infection based on detection of stage specific antigens in the serum and urine.

The specific objectives are to

1. analyse antigenic structure for the development of immunological reagents specific for B. malayi and W. bancrofti and those specific for various stages of the parasites.

2. devise simple, sensitive, specific, accurate and inexpensive immunoassays for the diagnosis of clinical lymphatic filaria as well as for the detection of early asymptomatic infection.

Work done to date

On visiting our project consultant, Dr. D.A. Denham of London School of Hygiene and Tropical Medicine, in April. The principal investigator had a good opportunity to discuss with the 'London Filariasis Group' which include Drs. R.M. Maizels, M.E. Selkirk of Imperial College of Science and Technology; Mr. R.R. Suswillo and his technicians working in the Department of Medical Helminthology, London School of Hygiene and Tropical Medicine. Discussion has also been made with Prof. R.E. Howells and Prof. W.W. Macdonald of Liverpool School of Tropical Medicine. The discussions were made in various topics which included the monoclonal antibody production, antigen characterization as well as problems in producing parasitic meterials. This will no doubt benefit the progress of our project.

In this period we failed to collect infective third stage larvae of Dirofilaria immitis which was attributed to the postponement of Physiology course in the Faculty. This course involves the use of dogs for experiments demonstrated to the medical students. The dogs used will be stray dogs obtaining from the Bangkok Municiple Dog Pound. More than 50% of these dogs are heavily infected with D. immitis, they will be a good source for collecting microfilariae, infective larvae and adults. The experiments will be postponed to early December 1988.

We also failed to raise polyclonal antibodies in rabbits and/or mice against the filarial and non-filairal antigens. We had a power failure in the Department during one week end in June and damaged some of our collections of antigens and antisera kept in the freezer. We are now expediting the re-collection of the spoiled materials.

Collection of parasitic materials and antisera

In addition to the parasitic materials and antisera that have

already been collected (listed in Table 1, 2, 3 of the progress report no. 1), we have collected microfilaria of Wuchereria bancrofti, fourth stage larvae of Brugia pahangi and their ES antigens, infective third stage larvae of Necator americanus, sera of 2 groups of normal people who have and never have experience of lymphangitis and lymphadenitis from the endemic areas of both B. malayi and W. bancrofti.

Microfilariae of W. bancrofti were obtained from blood of microfilaraemic patients living in endemic areas of the disease at the border line between Western Thailand and Burma. Heparinized blood was obtained from patients with microfilarial density of 300-500/60 ml peripheral blood. A volume of 1 ml of the heparinized blood was mixed with 10 ml of 0.1% NP-40 in PBS. The hemolysate was filtered through a nuclepore membrane, 25 mm in diameter, 5 μm poresize and microfilariae were recovered from the membrane by shaking in PBS and centrifuged at 1,000 rpm for 5 min. The larvae were washed once with PBS and the density was estimated under a dissecting microscope. The larval suspension in PBS was kept frozen at -20°C until use.

The fourth stage larvae of B. pahangi were obtained from the peritoneal cavity of jirds that had been injected with 100 L3s each for 10-14 days. The L4s obtained were cultured in NCTC 109 medium (DIFCO Laboratory, Detroit, Michigan) supplemented with 10% FCS incubated at 37°C in 5% CO_2 The medium was collected and replaced every day for around 14 days. The worms and the culture fluid were stored at -20°C.

Human feces containing hook worm (N. americanus) eggs was mixed with moist ground charcoal in a glass bowl and kept at room temperature (30°C) in shaded area for 10 days. The mixture was sprayed every day with water. On day 10, a gauze pad was placed on the charcoal and left the bowl exposed to light from a lamp for 1 hr to activate the larvae

to move upward to the gauze pad. The gauze pad was placed in a Baermann's apparatus filled with normal saline and the larvae collected from the bottom of the funnel were washed 2-3 times with normal saline and kept at -20°C.

Antigen characterization

The methods employed were as followed:

SDS-polyacrylamide gel electrophoresis

Vertical slab gel electrophoresis was performed in the presence of 1% sodium dodecyl sylfate as described by Laemmli (1970). Samples were prepared in sample buffer containing 0.625 M Tris, 1% SDS, 10% glycerol and 5% 2-mercaptoethanol and boiled in a boiling water bath for 2 1/2 min. They were then electrophoresed in a vertical slab gel consisted of a 10% SDS-polyacrylamide gel pH 8.9 together with a 4% stacking gel pH 6.8 using electrophoresis buffer pH 8.3. At the end of the electrophoresis, the gel was stained with 0.25% coomassie Brillient blue R or Silver stain (Merril et al., 1981). The gel was calibrated for molecular weight determination by running protein markers in parallel to the sample. The markers used were as followed: phosphorylase b (97,400), bovine serum albumin (66,000), egg albumin (45,000), trypsinogen (24,000) and lysozyme (14,300). All the markers, were purchased from "Sigma". Approximate molecular weights were determined by the method of Weber and Osborn , 1979).

Countercurrent electrophoresis

Sera obtained from animals experimentally infected with filarial parasites and those obtained from patients suffering from lymphatic filariasis were tested for the presence of antibodies against filarial antigens before using in radioimmunoprecipitation and immunoblot. Electrophoresis was performed in 1.5% agarose in barbitone buffer pH8.6

according to the technique previously described by Laurell (1965). A volume of 15 ml melted 1.5% agarose dissolved in barbitone buffer pH 8.6 was layered over a 8x10 cm^2 glas plate and allowed to solidify at room temperature. Fifty pairs of 2 mm diameter wells were made. The distance between wells of each pair was 5 mm. To run the electrophoresis, glass plates with the agarose gel were placed in a horizontal electrophoretic chamber containing barbitone buffer pH 8.6 having ionic strength 0.1. Two pieces of filter paper were used as bridges between the agarose and the buffer in the tank. Pre-electrophoretic running was performed by applying an electric current of 0.4-0.5 mA/cm width of the glass plate for 10-15 minutes in order to equilibrate and remove any salts from the agarose gel. Then equal volumes of 10 µl antigen and antiserum were applied in each pair of the wells. Electrophoresis was proceeded for 100 min at room temperature. After electrophoresis, nonreacted proteins were removed from the gel by soaking in several changes of 0.85% NaCl. The salt was then removed by soaking in water and the gel was dried at 37°C. Precipitin bands were stained with 0.6% Amido Schwartz 10 B.

1. Analysis of protein components in somatic extract of filarial worms

1.1 *Brugia pahangi* and *B. malayi*

Five adult female and 5 adult male worms of each *Brugia* species were homogenized together in 80 µl sample buffer and boiled for 2 1/2 min. A portion of 5 µl of each sample was electrophoresed in 10% SDS-polyacrylamide gel. After staining with coomassie brillient blue R, patterns of the protein compositions of the two *Brugia* parasites were compared. As shown in Fig. 1, adult *Brugia* worms are composed of at least 42 proteins of different sizes ranging from 180.5 kd to 13.0 kd.

They are composed of several major bands having estimated molecular weight around 150.0 kd, 46.0 kd, 35 kd and 14.3 kd. Although the protein patterns of these two species of *Brugia* worms looked similar, some differences are recognizable in the areas equivalent to approximate molecular weight of 93.0 kd, 70.0 kd, 64.0 kd, 25.9 kd and 18.5 kd. Further analysis of antigenic property of these proteins is to be carried out.

1.2 *Wuchereria bancrofti*

Soluble aqueous extract of *W. bancrofti* microfilariae was subjected to 10% SDS-polyacrylamide gel electrophoresis and stained with silver nitrate staining. As shown in Fig. 2, the somatic extract of microfilariae are composed of at leas 44 proteins of different sizes ranging from 95.0 kd to 13.0 kd. Six major components were demonstrated to be proteins having estimated molecular weight of 72.5 kd, 47.5 kd, 33.5 kd, 26.5 kd, 16.5 kd and 13.0 kd. When comparing to the extract of white blood cells obtained from normal human, several protein compoments in the microfilariae extract were found to have molecular sizes equivalent to those in white blood cell preparation (see Fig. 3). It is not known at this stage whether these components are the proteins belonging to the white blood cells contaminated into the microfilarial preparation or they are the protein of the parasite that share the same characteristic with those belonging to the hosts' white blood cells.

Comparison between the soluble aqueous extract of *W. bancrofti* microfilariae and that of adult *B. malayi* was also performed. As shown in Fig. 3, the gel stained with silver nitrate staining showed the existence of proteins having the same molecular sizes in both the somatic extract of *W. bancrofti* microfilariae and adult *B. malayi*. Sharing proteins of equivalent molecular sizes among these two worms and human blood cells were also demonstrated in the same gel.

2. Detection of precipitating antibodies against Brugia antigens in antisera

2.1 Infected animal sera

Antisera obtained from animals experimentally infected with *Brugia* parasites was examined for the presence of precipitating antibodies against *Brugia* antigens by countercurrent electrophoresis. This experiment was performed in order to select for the positive control antisera to be used for antigen characterization of our filarial worms. The antisera used are listed below:-

a) Antiserum obtained from microfilaraemic cats infected with *B. malayi*.

b) Antiserum obtained from jirds intraperitoneally infected with *B. pahangi*. Microfilariae were found in the peritoneal cavity when autopsied after blood collection.

c) Antiserum obtained from amicrofilaraemic cats that had been infected with several doses of *B. pahangi* infective larvae.

d) Antiserum obtained from jirds infected subcutaneously with *B. pahangi* infective larvae that had been irradiated with gamma ray from ^{60}Co source at 45 krad.

e) Antiserum obtained from jirds infected subcutaneously with *B. malayi* infective larvae that had been irradiated at 25 krad.

f) Antiserum obtained from jirds intraperitoneally infected with *B. malayi* infective larvae. Microfilariae were found in the peritoneal cavity when autopsied after blood collection.

The reaction was also performed with three different sera collected from non-parasitized animals and human in order to select for the negative control sera. The three sera are listed below:-

g) Normal cat serum.

h) Normal jird serum

i) Normal human serum

As shown in Fig. 4, all 6 antisera obtained from infected animals reacted with the somatic extract of B. pahangi giving precipitin bands that are clearly visible after staining with Amido black. The 3 normal sera did not react with the Brugia antigen.

2.2 Infected human sera

Sera obtained from patients suffering from lymphatic filariasis in Thailand were tested for the presence of precipitating antibodies against Brugia antigens. The sera collected were from different groups of patients that can be categorized as follows:-

a) B. malayi infected patients showing elephantiasis of the leg(s). They are all amicrofilaraemic.

b) W. bancrofti infected patients suffering from hydrocoele for 1-10 years. All are amicrofilaraemic.

c) People living in the endemic area of B. malayi who had experience of lymphadenitis and lymphangitis but show no symptoms at present time. They had never been found to be microfilaraemic.

As shown in Fig. 5 an 6, most of the sera obtained from amicrofilaraemic patients infected with B. malayi reacted strongly to the somatic extract of B. pahangi adult worms. Sera obtained from W. bancrofti infected patients did not react to the B. pahangi antigen in this experiment. It was clearly demonstrated that persons who had been suffering from lymphadenitis and lymphangitis in the past developed strong antibody response against the parasite antigen (See Fig. 6, S_3h-j, S_4 and S_5).

Production of Monoclonal Antibodies:

We have begun producing hybridomas with our most abundant antigen, total somatic antigen from *Brugia pahangi*. This will enable us to work out immunization and assay schemes and train a technical assistant in routine aspects of hybridoma production concurrently with our ongoing characterization of filarial antigens using polyclonal antibodies. Once we have a clearer picture of exactly which specific antigens we wish to identify through monoclonal antibodies, our efforts will be refocused to concentrate on those antigens of greater interest and utility. To date, we have done one successful fusion using *B. pahangi* antigen.

Somatic antigen was prepared by homogenizing adult worms hand-picked from peritoneal cavities of experimentally infected jirds. Male and female worms were pooled separately and homogenized in small volumes of sterile saline with 0.2 mM PMSF. The homogenates were sonicated briefly before centrifugation, and a portion of each was analysed for total protein concentration by a modified (Bradford) Coomassie Blue G binding assay. Extracts from males were approximately 10-fold lower protein concentration. The 2 extracts were mixed in roughly the same recovery ratio (*i.e.* equal vol. not equal conc.) for immunization and ELISA plate coating.

A series of BALB/c mice were immunized with antigen spotted and adsorbed onto nitrocellulose sheet. The adsorption appeared to stimulate good immune responses with minimal amounts of antigen, as reported previously in the literature. The nitrocellulose-antigen was dissolved in DMSO and mixed with 2 vol Freund's complete adjuvant before i.p. and s.c. injection (the mouse used in the fusion received less than 15 μg Ag at this stage). The Ab responses were assayed after 3 wks, and the animals were boostered i.p. with 25 μg of similarly adsorbed Ag but

in incomplete adjuvant at 4 wks. The best responder reassayed by ELISA after 3 wks was given a "pre-fusion booster" consisting of 100 µg soluble Ag injected i.p. on day 60, and was used in the fusion 3 days later.

Spleen cells from this immune mouse were fused with 653 myeloma cells using PEG fusogen and standard procedures. The fusion mixture in HAT selection medium was distributed together with splenocyte feeder cells in 10 plates. Wells were re-fed with HT medium on day 8 and the fastest growing wells were assayed by ELISA on day 12. All wells were re-assayed on day 15 and cells from the strongest + wells were transferred to 24-well plates. The fastest growing "mother" colonies are just now ready for back-up freezing and 1st cloning.

Electron microscopic studies

1. The structure of cuticle and body wall of microfilaria and infective larvae of Brugia malayi and Brugia pahangi

Before embarking upon the immunofluorescence and immunocytochemical studies of antigen-antibody interaction on the surface of the parasites, we have performed basic studies of the formation and organization of the cuticle and body wall of microfilariae and infective larvae of two species of parasites as mentioned, using both scanning and transmission electron miroscopies.

1.1 The formation of sheath and cuticle of microfilaria of Brugia pahangi (Figure 7)

B. pahangi embryo is sheathed by the egg shell (Fig. A, arrow). The cuticle is very thin and composed of electron dense outer membrane separated from the inner membrane by thin fibrous zone. Basal lamella separates the cuticle from the hypodermis which is the cytoplasmic sheet

of hypodermal cells (Fig. B, arrow head). These cells contain microtubules (Mt), mitochondria and large membrane bound vesicle (MBV). Microfilaria of B. pahangi is surrounded by a sheath (Sh) whose surface appears granular while the surface of microfilaria itself is annulate (An) (Fig. C). The sheath is composed of two layers: the outer dense layer underlined by pale homogeneous layer. The space between body and sheath is filled with flocculent material. The cuticle of microfilaria is thin and composed of the external cortical (EL), the internal cortical (IL) and the fibrous layers (FL) (Fig. D-F). The outermost external cortical layer is most electron-dense and is often discontinuous at the annular grooves. The middle internal cortical layer is moderately dense. The innermost layer which is the thickest in cuticle, is composed of fibrous components. Hypodermis (hy) is situated beneath the fibrous layer of the cuticle and grouped into narrow dorsal, ventral and broad lateral cords. Adjacent hypodermal cells are connected by desmosomes. The hypodermis contains nucleus (Nu), mitochondria (Mi), microtubules (Mt), ribosome (Ri) and a dense amorphous bodies (DB) of particular interest are microtubules which "spray" into the thin layer of hypodermis underlining the cuticle (Fig. D-F). The muscle cells (Mu) are arranged in four groups except at the head and the tail. Each cell is composed of a contractile and noncontractile portion. The contractile portion adjacent to the hypodermis contains many thick and thin myofilaments. The noncontractile portion, which has mitochondria, nuclei, a number of glycogen particles and other organelles, is located in the inner part of the cell.

1.2 The body wall of the infective larvae of Brugia malayi and Brugia pahangi (Figure 8)

The body wall of the infective larva of B. malayi (Fig. A, C &

E) and B. pahangi (Fig. B, D & F) consist of cuticle (Cu), hypodermis (Hy) and somatic musculature (Mu) (Fig. A-D). The cuticle comprises three layers: the densely stained epicuticle (Ep), exocuticle (Ex), and the thick finely fibrous layer (FL) (Fig. C & D). The cuticles of two species can be distinguished by the presence of dense striated band in the middle of fibrous layer in B. pahangi but not in B. malayi (Fig. F, arrow head). The hypodermis appears as a thin layer beneath most of the cuticle except in areas of hypodermal cords (A, B & D). The larval hypodermis follows the usual nematode pattern with the four cords separating the muscle quadrants. The dorsal and ventral cords are pedunculate ingrowths of the hypodermis whose cells contain numerous mitochondria, ribosome RER, Golgi bodies and some microtubules (MT), especially at the cytoplasmic area in contact with cuticular region (Fig. C). This characteristics indicates high levels of synthetic activities of cuticular formation. Nuclei of both the dorsal and ventral cords have large prominent nucleoli and electron lucent-chromatin. Neural elements are particularly prominent in the ventral and to a lesser extent, in the dorsal cords. The lateral cords comprise three interconnecting but separately nucleated sectors, one smaller median (MLS) and two larger sublateral (SLS) (Fig. E). The lateral cords are broadly attached to the cuticle and protrude into the pseudocoel withou pedunculation. Sectorial boundaries are distinct, with the two sublateral sectors enclosing the medial sector. Tight junction between the sectors occur in the immediately subcuticular area of the medial sector (Fig. E, arrow head). Although we cannot differentiate the surface structure among both species under SEM, the longitudinal striated dense band in fibrous layer was observed only in B. pahangi under TEM (Fig. F, arrow head).

References

1. Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (London), 227, 680-685.
2. Laurell, C.B. (1965). Antigen-antibody crossed electrophoresis. Analyt. Biochem., 10, 358-361.
3. Merril, C.R., Goldman, D., Sedman, S.A. and Ebert, M.H., (1981). Ultrasensitive stain for protein in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science, 221, 1437-1438.
4. Weber, K. and Osborn, M., (1969). The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem., 244, 4406-4412.

Work plan for the next period

1. Continue collecting parasitic materials and antisera which will be a routine work until sufficient amounts for the experiments are obtained.

2. Raise polyclonal antibodies in rabbits and/or mice against each of the antigenic preparations of filarial and non-filarial parasites.

3. Monoclonal antibodies:

Hybrid cells in 24-well plates must be cloned and sub-cloned to ensure mono-clonality, and frozen stocks of sub-clones set up. We will also begin to characterize the fine specificities of the monoclonal antibodies by Western blot or radioimmunoprecipitation. The remaining animals primed and once boostered with *Brugia pahangi* total adult somatic antigen will be re-boostered at 2-month intervals to hopefully increase Ab affinities should these be needed for additional fusions.

We hope we will be able to start the immunization with additional, or perhaps more refined antigens.

4. Analysis of filarial antigens, i.e. somatic extract of adult B. pahangi and B. malayi and somatic extract of W. bancrofti microfilariae by SDS-polyacrylamide gel electrophoresis follow by Western blot and immuno-enzyme assay against positive control sera and sera from patients suffering from lymphatic filariasis.

5. Perform the immunofluorescence and immunocytochemical studies to localize the antigens that elecit the antibody formation in hosts. Whole sera and/or fractionated IgG of both infected animals and human patients will be used to localize the surface and excretory antigens at light microscopic level by using indirect immunofluorescence technique (using second antibody labelled with FITC). In addition, protein A labelled with horse radish peroxidase (HRP) will also be used in immunocytochemical experiment at both light and electron microscopic levels. By employing these techniques we hope to be able to localize sites of antigens in the parasites bodies that bind to natural antibodies from the sera of infected hosts.

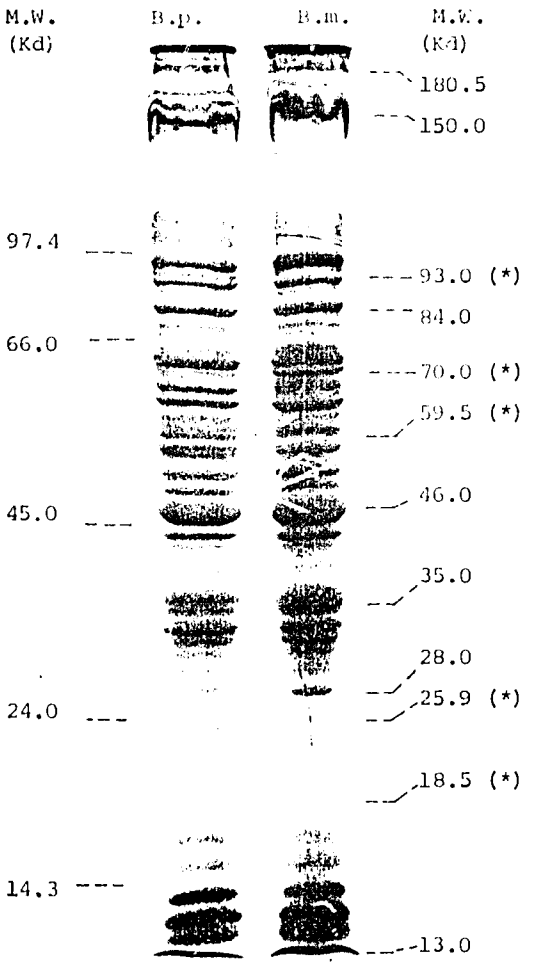


Fig. 1. Brillient blue R staining of whole adult Brugia pahangi (B.p.) and B. malayi (B.m) extracts in 10% SDS-polyacrylamide gel. Differences in protein profile of these two worms are demonstrated (*).

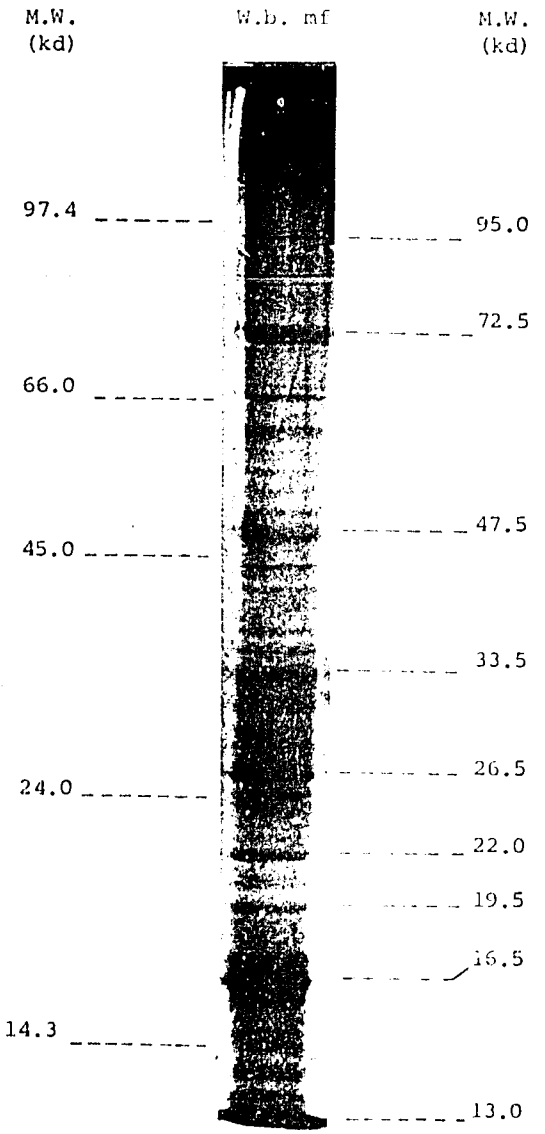


Fig. 2. Silver staining of aqueous soluble extract of *Wuchereria bancrofti* microfilariae (W.b. mf) in 10% SDS-polyacrylamide gel.

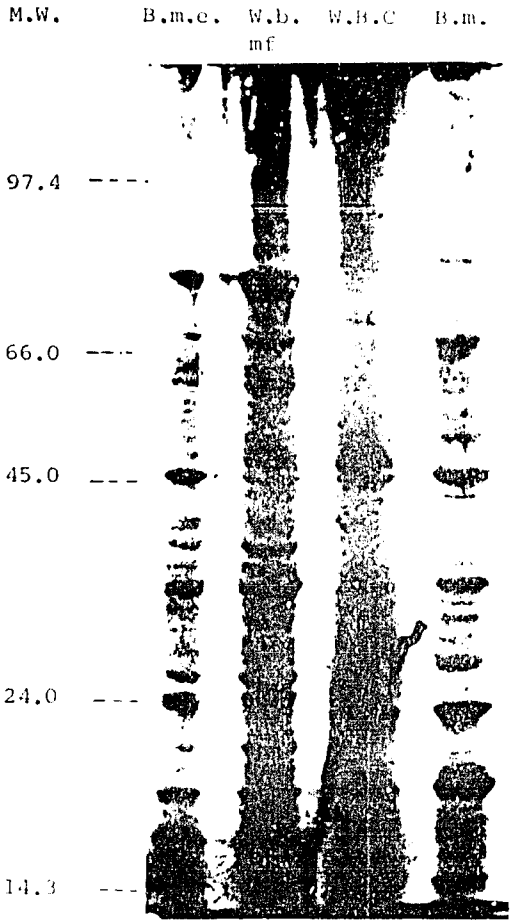


Fig. 3. Silver staining of soluble aqueous extracts of adult *Brugia malayi* (B.m.e.), microfilariae of *Wuchereria bancrofti* (W.b. mf) and normal human white blood cells (W.B.C.) in 10% SDS-polyacrylamide gel.

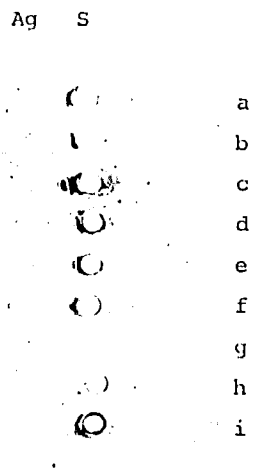


Fig. 4. Amido black staining of a countercurrent electrophoresis of aqueous soluble extract of adult *Brugia pahangi* against positive control sera from *B. malayi* infected cat (Sa) *B. pahangi* infected jird (Sb), *B. pahangi* infected cat (Sc), 45 krad-irradiated *B. pahangi* infected jird (Sd), 25 Krad irradiated *B. pahangi* infected jird (Se), *B. malayi* infected jird (Sf) and negative control sera from normal cat (Sg), normal jird (Sh) and normal human (Si).

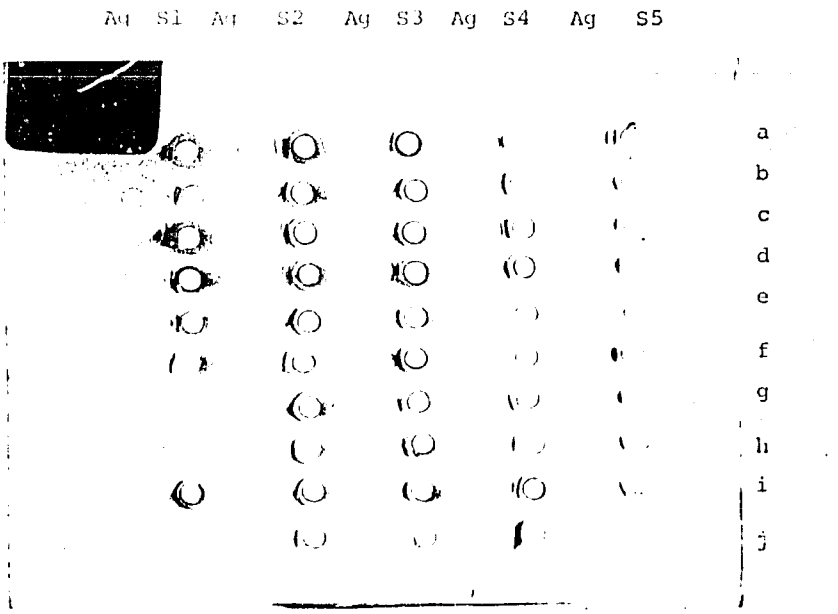


Fig. 5. Amido black staining of a countercurrent electrophoresis of aqueous soluble extract of adult B. pahangi (Ag) against positive control sera (S_1a-f), negative control sera (S_1g-i), sera from symptomatic amicrofilaraemic patients infected with B. malayi from Southern Thailand (S_2-S_5).

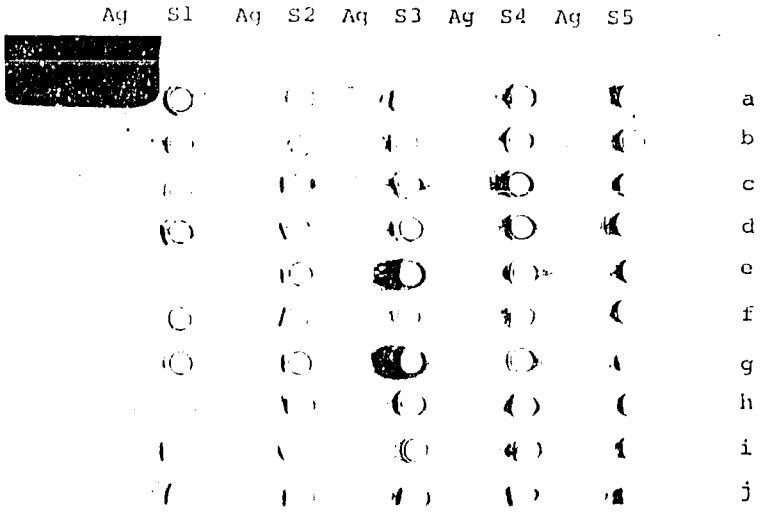
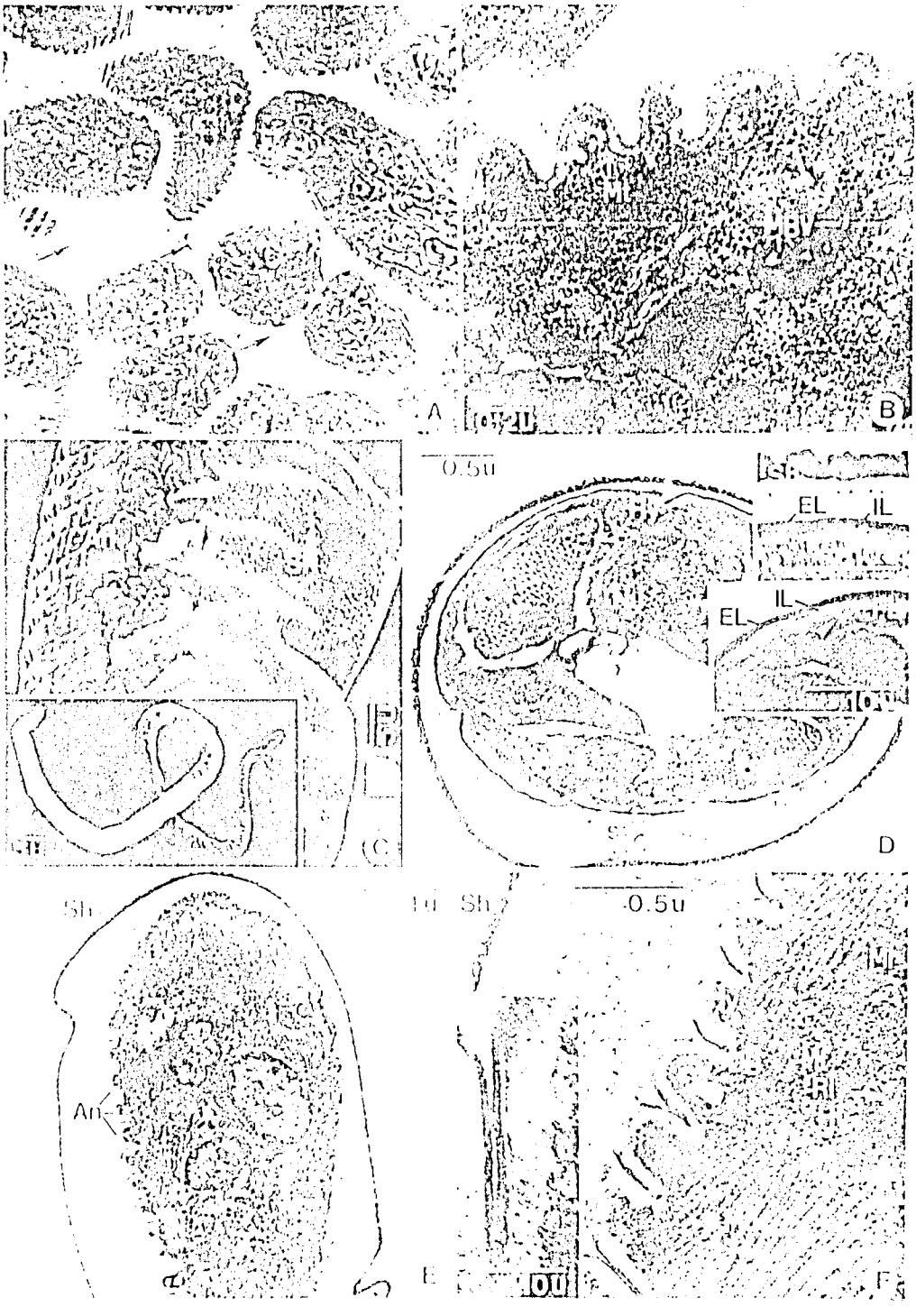
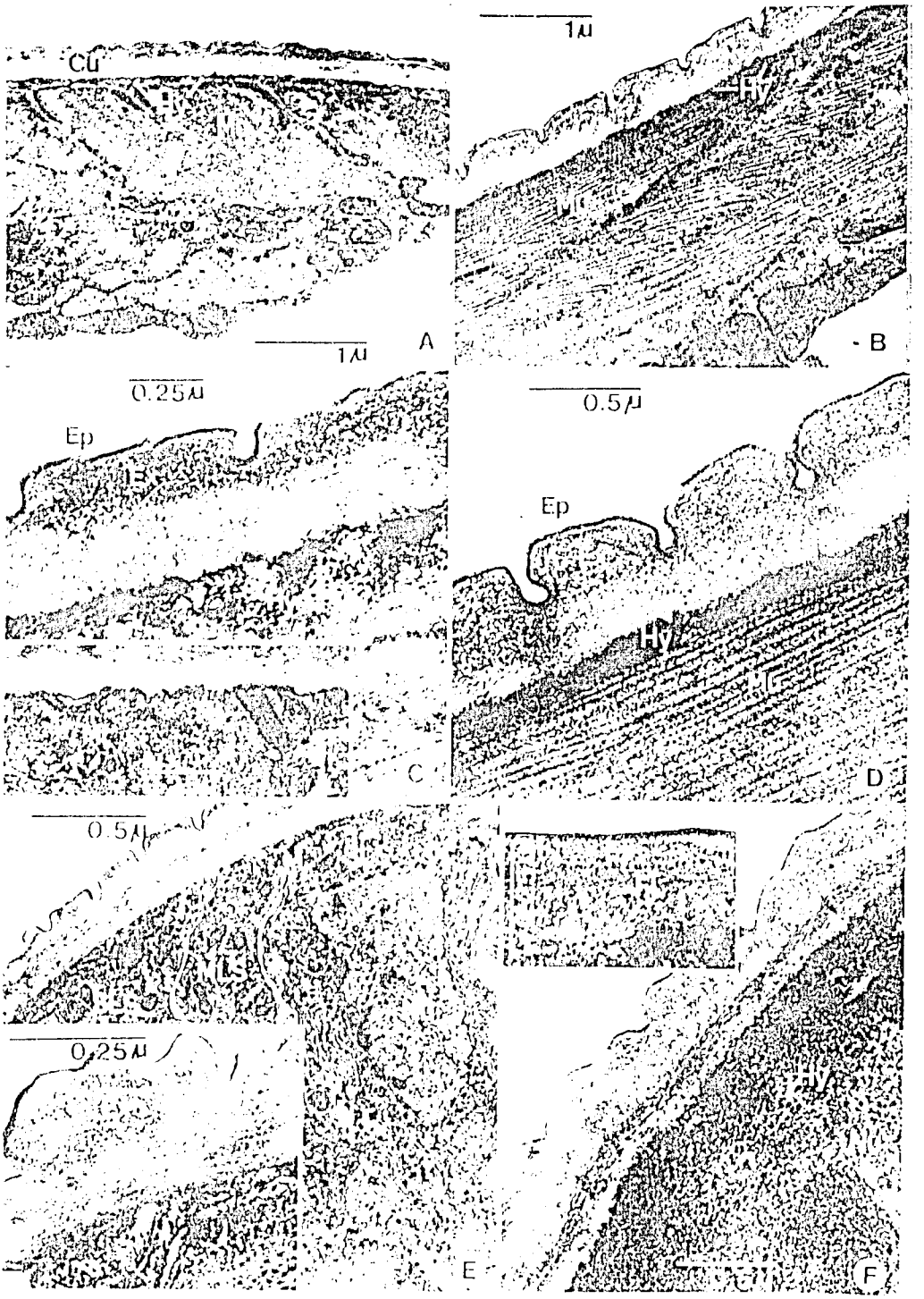


Fig. 6. Amido black staining of a countercurrent electrophoresis gel of aqueous soluble extract of adult *B. pahangi* (Ag) against sera from symptomatic amicrofilaraemic patients infected with *B. malayi* from Southern Thailand (S_1, S_2, S_3a-d), sera from symptomatic amicrofilaraemic patients infected with *W. bancrofti* from Western Thailand (S_3e-g) and sera from normal persons who had experience of lymphadenitis and lymphangitis from the endemic area of *B. malayi*, Southern Thailand (S_3h-j, S_4 and S_5).


A
B
0.5u
EL
IL
EL
IL
C
D
Sh
Sh
0.5u
An
E
F


Cu
1μ
A
1μ
Hy
B
0.25μ
Ep
C
0.5μ
Ep
Hy
D
0.5μ
0.25μ
E
F