ビルマ国感染症研究対策プロジェクト
エバリュエーション調査団報告書

昭和57年4月

国際協力事業団
医療協力部

ビルマ国感染症研究対策プロジェクト
エバリュエーション調査団報告書

昭和57年4月

国際協力事業団
医療協力部

はしがき

ビルマ国感染症研究対策プロジェクトは、日本政府の無償資金協力により1980年3月に完成した生物医学研究センターの施設を活用し、同国の感染症に対する生物医学的研究の発展に資することを目的に、1980年4月10日に署名された討議議事録に基き、2年間の協力期間をもって開始された。

この2年間における専門家派遣、研修員受入および機材供与等の協力により、研究の各分野において一定の進歩が見られ、本プロジェクトの役割が益々重要になってきたことから、ビルマ国政府は更に2年間の協力延長を要請してきた。

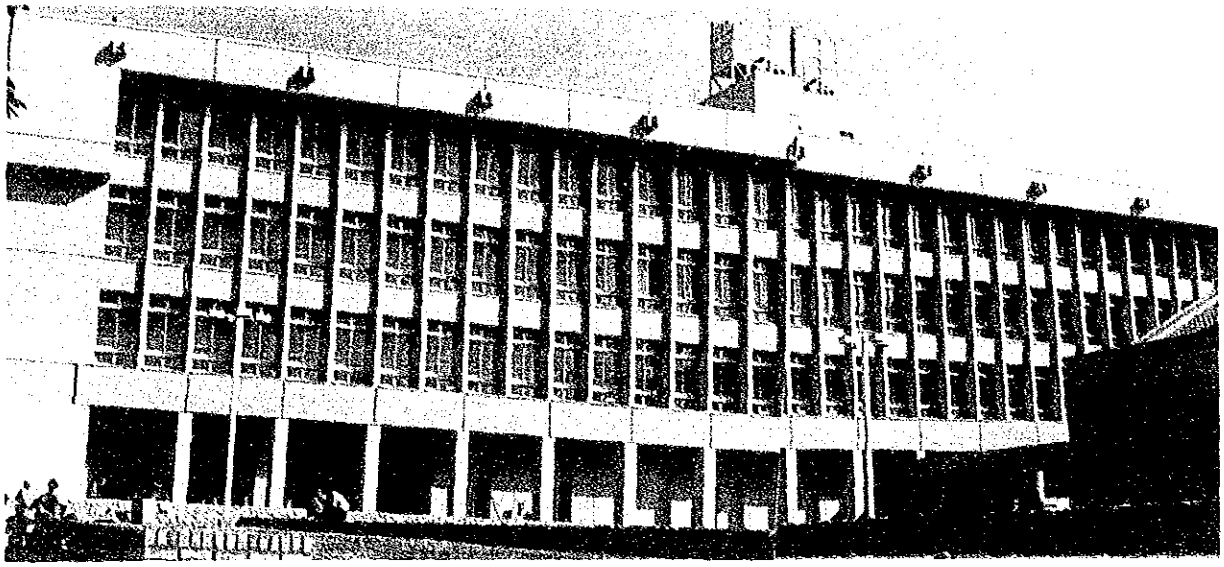
以上の経緯から、今般、エバリュエーション調査団を派遣し調査した結果、協力期間延長が必要かつ妥当であるとの結論に達したので、討議議事録に署名し、引続き協力事業を実施することとなったものである。

ここに調査団団員の各位ならびに調査団の派遣にご協力を賜った関係機関の各位に、深甚なる謝意を表するとともに、本プロジェクトの今後の運営について一層のご協力を賜りたくお願いする次第である。

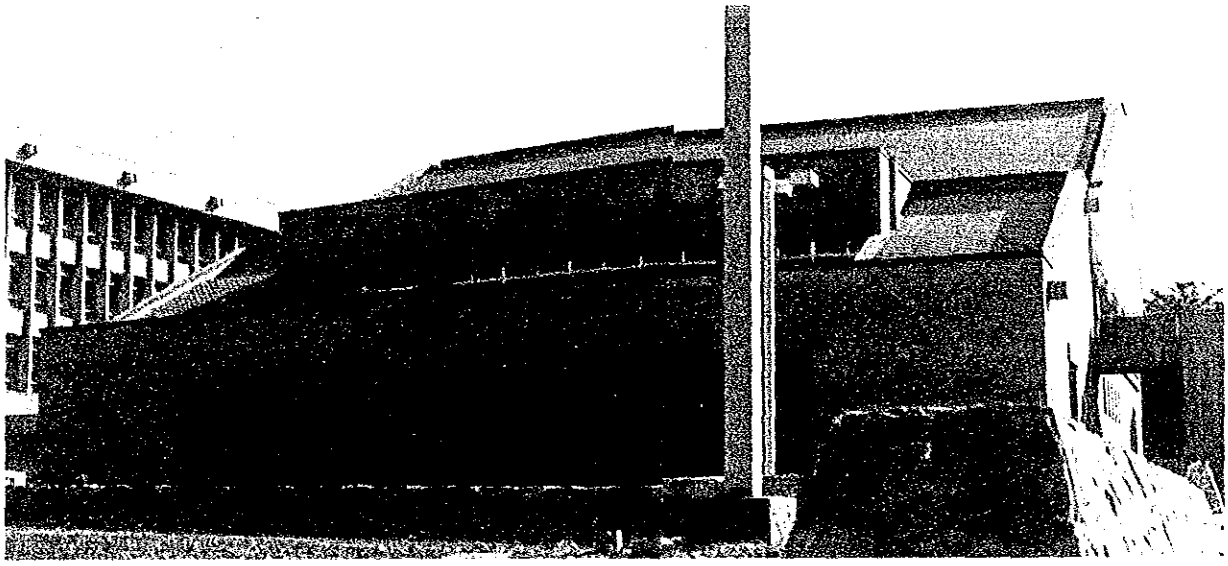
昭和57年4月

国際協力事業団

理事　長谷川　正　男



生物医学研究センター



同　医学図書館

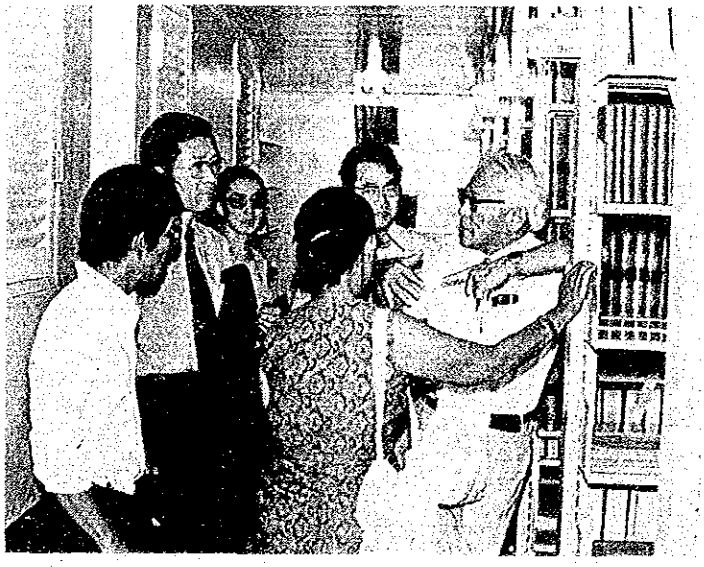
Coordinating Committee 座長
Col. Tun Hla Pru 副大臣の挨拶

日本側：エバリュエーション
調査団と日本大使館員

副大臣と濱島団長

Committee 後の座談会（左から吉田団員・早川公使・副大臣・濱島団長・AUNG THAN BATU局長・KYWE THE IN副局長）

ウイルス部門見学の吉田団員・武田所長



医学図書館内見学

目　　次

Tentative Programme for JICA Evaluation Team (17 - 24 Feb, 1982.)

Date / time			Participants	Place	Remarks
17 Feb. Wed.	p.m.	Arrival. Professor Y. Hamashima and Professor O. Yoshida	-	-	Director General and others to meet at airport
18 Feb. Thurs.	9:00)	i. Meet with Director General	Team and Director General	V.I.P. Room	
	9:15) a.m.	ii. Arrival of Deputy Minister	-	V.I.P. Room	
	9:30)	iii. Coordinating Committee Meeting	Deputy Minister, Director-General + Committee-Member + Evaluation Team.	Conference Room	Distribution of Report.
		iv. Departure of Deputy Minister			
		v. Presentations to Evaluation Team by Burmese Scientists	Evaluation Team, Burmese and Japanese Scientists.	Conference Room	Projectors, Microphone etc.
	p.m.	vi. Continuation of Presentations and discussion on them.	- ditto -	- ditto -	
19 Feb. Fri.	a.m.	i. - ditto -	- ditto -	- ditto -	
		LUNCH BREAK			
	p.m.	ii. - ditto -	- ditto -	- ditto -	
20 Feb. Sat.	a.m.	i. Evaluation Team to go around Research and other Division.	Deputy Director, Daw Mi Mi Khin, Team and relevant Scientists.	Division	Division to prepare Assistant Director, to prepare.
	p.m.	HOLIDAY			
21 Feb. Sun.		HOLIDAY			
	p.m.	Arrival of Dr. Nakazawa	-	-	Director General and others to meet at Airport

Date / Time				Participants	Place	Remarks
22 Feb. Mon.	a.m.	i.	Over view of Project	Evaluation Team + Burmese and Japanese Scientists.	Conference Room	
		ii.	Discussion on future scientific programme.	- ditto -	-	
	p.m.	iii.	Coordinating Committee Meeting Arrival of Miss Kamijo	Coordinating Committee Members + Evaluation Team.	-	
23 Feb. Tue.	a.m.	i.	Visit to field practice area + Research Division	Evaluation Team + Japanese and Burmese Scientists + Deputy Director Daw Mi Mi Khin	North Okkalapa	
		ii.	Final discussion with Director General.	Director General + Evaluation Team.	Director General Office	
24 Feb. Wed.	a.m.		Departure of Team Leader & Dr. Yoshida			Seen off by Director-General, and other at Airport.
25 Feb. Thur.	p.m.		Departure of Dr. Nakazawa & Miss Kamijo			

Note: Sessions - (9:30 a.m - 12:30 p.m)
(2 p.m - 4 p.m)
Coffee break about 10:30 a.m -)
Tea about 3 p.m -) - Except 18th when it will be earlier.

* HTUN/-

1. エバリュエーション調査団派遣の経緯とその目的

本プロジェクトは昭和５５年４月１０日から昭和５７年４月９日までの協力期間において、ビルマ国の主要アルボウイルス性疾患および主要細菌性腸管疾患の研究を行い、その成果を１モデル地区における疾病対策に応用することを目標として協力を実施してきたものである。

協力の対象機関としてはビルマ国保健省医学研究局を中心とし、日本政府の無償資金協力により建設された生物医学研究センターの施設を利用して研究・協力活動が実施された。

これまでの協力により、ビルマ側の研究体制の確立、要員の養成にも効果があがっていることが予想されるとともに、今次Ｒ／Ｄ期間が昭和５７年４月９日に了することに伴い、これまでの協力の成果の評価ならびに協力期間の延長の可否を検討する目的をもって、本件エバリュエーション調査団を派遣することとしたものである。

なお、調査の結果、更に協力期間の延長が必要と判断される場合には、その協力の方法について協議を行い、協力の基本計画を作成の上、これを討議議事録にとりまとめ、署名することについても調査団に付託し、派遣したものである。

2. エバリュエーション調査団の編成

団　　長　　濱　島　義　博
京都大学医学部（病理学）教授

団　　員　　吉　田　　修
京都大学医学部（泌尿器科）教授

団　　員　　中　澤　幸　一
国際協力事業団医療協力部長

団　　員　　上　條　三津代
国際協力事業団研修事業部

3. エバリュエーション調査団日程

（昭和５７年２月１６日～２６日）

時間 / 月日	午前	午後	備考
2月16日(火)	11:30 成田発ＪＡＬ４６３	17:30 バンコック着	
2月17日(水)		13:40 バンコック発 バングラデシュ航空７３ 14:30 ラングーン着	
2月18日(木)	7:00 専門家meeting 9:30 医学研究局にて Coordinating Committee 保健副大臣Col. Tun Hla Pru座長をつとめる 10:00 エバリュエーション調査団に対して研究発表ならびに討論 （細菌学部門）	13:00 研究発表継続 （ウィルス学部門） 16:00 Dr. Aung Than Batu 局長と打合せ	
2月19日(金)	7:00 専門家meeting 9:30 研究発表継続 （免疫学部門、病理学部門）	14:00 研究発表継続 （プロジェクト外研究発表）	
2月20日(土)	7:00 専門家meeting 9:30 生物医学研究センター内見学		
2月21日(日)	休　日	19:00 医学研究局スタッフと研究局中庭にて夕食会。 中沢部長到着。	
2月22日(月)	7:00 専門家meeting 9:00 日本大使館訪問。 早川公使、本田書記官、武田ＪＩＣＡ駐在員と打合せ 9:30 Coordinating Committee Meeting （医学研究局にて）	13:00 午前の継続 14:00 ビルマ医学研究展望の討論会 16:00 上條団員到着	

時間 / 月日	午前	午後	備考
2月23日(火)	9:30 プロジェクト延長手続きの審議 11:00 北オカラッパ地区見学	12:30 団長日本大使館早川公使とR／D延長原案審議 13:30 minutes に署名 14:00 ビルマ側研究成果大要報告ならびに討論会 18:30 ビルマ側関係者を招待して団長主催の夕食会	
2月24日(水)	9:30 中沢部長、上條団員はビルマ製薬研究所訪問	14:30 団長と吉田教授両名はラングーン出発(UBA202) 16:00 バンコック着	
2月25日(木)	9:00 バンコック発TG610（団長、吉田）	16:00 中沢部長、上條団員ラングーン発TG306 17:40 中沢部長、上条団員バンコック着 19:15 団長、吉田教授帰国	
2月26日(金)	8:30 中沢部長、上條団員バンコック発JL474	16:05 中沢部長、上條団員帰国	

4. 関係者氏名

(1) 日本大使館

橘　正忠大使　　早川照男公使、

本田均　一等書記官、武田慶一ＪＩＣＡ所長

(2) ＪＩＣＡ派遣専門家

中根一穂東海大学（病理学）教授

林英生岡山大学医学部（細菌学）助教授

伊藤富由関西医大（微生物学）講師

浅野敏彦予防衛生研究所所員

(3) ビルマ保健省

保健大臣　U Tun Wai

保健副大臣　Col. Tun Hla Pru

医学研究所

局長　Dr. Aung Than Batu

副局長　Dr. Kywe Thein

副局長　Dr. Khin Maung Tin

副局長　Dr. Mi Mi Khin

Virology

Dr. Soe Thein

Dr. Kyi Kyi Khin

U Thet Win

Bacteriology

Dr. Tin Aye

Daw Mar Mar Nyein

Immunology

Dr. Tun Pe

Dr. Khin Ohn Lwin

Experimental Medicine

Dr. Hla Myint

Nutrition

Dr. Cho Nwe Oo

Hematology

Dr. Thein Toe

Dr. Khin Maung Oo

Epidemiology

Dr. Thein Maung Myint

Instrumentation

U Soe Myint

Entomology

Mr. A. A. Sebastian

Experimental Animal Centre

U Khin Maung Zaw

Library

Daw Hla Kyi

5. 調　査　内　容

ビルマ国感染症プロジェクトに対する協力の評価

§ 評価の目的：

ビルマ国感染症プロジェクトに対する技術協力は、1980年4月10日にサインされたR／Dによって2年間の期間の予定で開始された。これは1980年3月に完成した日本国外務省経済協力無償供与による生物医学研究センターの指導協力実施に関するプロジェクトである。この生物医学研究センターはその設立の主旨なるものが“ビルマ連邦社会主義共和国における生物医学研究の促進を図り、基礎医学研究分野の一層の発展を期し、日緬両国の長年にわたる協力関係の成果として設立されたものであり、今後の日緬両国の生物医学分野における協力関係の礎となることを確信するものである”と示されていることから、今回のエバリュエーションの目標としてこれを基本理念と定めその成果の実体を具体的に評価することを目的とした。

§ 第1次Coordinating Committee Meeting

ビルマ国感染症プロジェクトの過去2カ年にわたる研究、運営、友好などの成果を評価するに先立ち、ビルマ側は、R／D（5頁、VI,Administration of the project, 2ならびに12頁、Annex VI Composition of the Coordinating Committee）にもとづいた、保健副大臣を座長とする。Coordinating Committee Meeting を開催することからこのたびのJICA evaluation teamの活動が開始された。

2月18日午前9時30分より、生物医学研究センター、カンファレンスルームにおいて、保健副大臣Col. Tun Hla Pru の座長によって本Meeting が開かれた。日本側からは、調査団団長と吉田修京大教授、日本大使館側からは早川照男公使、本田均一等書記官、武田慶一ＪＩＣＡ所長、ＪＩＣＡ専門家として中根一穂東海大学教授、林英生岡山大助教授、伊藤富由関西医大講師、浅野予研究員。ビルマ側からは医学研究局オンタンバツー局長、ジョーテイン副局長、キンマウンティン副局長、ミミキン副局長、ならびにＪＩＣＡプロジェクトに関係ある各部主任全員総勢19名が参加した。

座長の保健副大臣の示された要旨は次のものであった。

1. What has been accomplished under the JICA/DMR Project up to now?

   a. We have initiated scientifically sound Research Programs in Bacterial Enteric Diseases and Arboviral Diseases.

   b. Already, Research Results have been obtained which are of high scientific quality and of great use for application to the Control of these Diseases.

      This is especially note-worthy in Bacterial Enteric Diseases

Research.

A National Research Seminar on Diarrhoea Diseases is scheduled to be held on 27th February, Saturday - Which will be attended by Professors, top Pediatricians and top Public Health authorities - at which the Research Findings on Bacterial Enteric Diseases of the JICA/DMR Project - will be communicated so that the result will be quickly utilized by these key people for Control of Bacteric Enteric Diseases and in Teaching.

c. The infrastructure necessary for good quality and useful research in Bacterial Enteric Diseases and Arboviral Diseases has been firmly established.

d. Considerable development in laboratory technology has taken place.

e. A vitally important but intangible achievement is the cross-fertilization of ideas and stimulation which has taken place in whole of DMR on a consequence of the implementation of this Project.

2. What remains to be done within the context of the aims of the Project?

a. More in-depth research will be required in Bacterial Enteric Diseases.

b. Research into DHF - which has just started - needs to be carried out full-scale.

c. Development of technology, of course, has no end but establishment of Hybridoma technology and establishment of technology for the study of ultrastructure, clotting disorders, and micro-circulation is further required.

In conclusion I should like to state that Japanese scientists and Burmese scientists and technicians have worked together in a spirit of good-will and mutual respect.

I am confident that, with further collaboration in this manner, we will acheive all the aim of the Project.

以上のようにこの2月18日午前9時30分より行われた第一次 Coordinating Committee Meeting では、座長である副大臣による過去2カ年に及ぶＪＩＣＡプロジェクトの経過報告と将来の要望の説明があり、議題らしいものは何一つ提案されずに約30分で解散。本格的な討議は改めて2月22日に、中沢部長参加のもとに2回目の Coordinating Committee Meeting を開き、そこで今後の方針などの討議を行うということになった。

しかし基本的には、このプロジェクトの将来についての論議は、エバリュエーション調査の結果が出たあとで討議するという形は保持された。

§ 研究成果の発表会とその成果（後章英文Research Findings ,Repoet 参照）

2月18日午前10時30分より、エバリュエーション調査団に対する各部局の過去2年間におけるＪＩＣＡプロジェクトの研究成果の発表会が開始された。これは翌2月19日午後4時過ぎまで続いたかなり活発な研究会であった。

2月18日　午前

細菌学部門

1. 急性下痢症起炎菌の同定法の研究　Dr. Daw Tin Aye（細菌部主任）。

本研究は、5才以下児童の急性下痢症の主要病原体の同定と、児童間起炎菌伝播の疫学的観察の2点を目的として実施されたもので1981年には、乾季に407例、雨季に1545例の児童下痢症について検討した。その結果、雨季では3才以下に多く、乾季では両者に大きな差は見られず、病原菌としては毒素原性大腸菌29.9％、病源菌12.0％、志賀赤痢菌3.6％が主たるもので、サルモネラやコレラ菌は予想に反して極めて少数であった。技術の顕著な向上によって、病原菌の分離率は80％という高率、好成績を挙げるに到っている。

2. 北オッカラパ地区児童急性下痢症の流行とその原因　Daw Mar Mar Nyein

1980、1981 両年にわたり乾季と雨季両者の5才以下児童の下痢を24地区より407例（乾季）、1545例（雨季）を蒐集、主としてその起炎菌の同定と分類ならびに分離を試みた。

その結果5才未満児急性下痢症は乾季で16.7％、雨季で20.3％と大差なく、年令は雨季では生後1才～2才児に断然多い。

下痢症の原因は細菌性とウィルス性とに分けられ、細菌では、病原性大腸菌、サルモネラ、志賀赤痢菌、これにカムピロバクターとエルシニアとが挙げられる。今回の調査で、ビルマで

現在、８０％の高率で下痢症の原因菌を確定することが出来るようになった。

2月１８日　午後13:30～17:00

ウィルス学部門研究発表

1. 蚊脳内接種法によるデングーウィルスの新しい迅速法の開拓

Dr. Daw Mi Mi Khin（副局長）

U Thet Win

1980年の流行期において６３例の非ショックデングー熱患児と、５例のショックデングー熱患者の末梢血より単球細胞群（Ficoll-hypaque分離法による）を集め、これを蚊の頚背部接種を行ったのち、３２℃、８０％湿度の条件で１０％の砂糖液で飼育しその５日目の蚊の頭部磨滅標本内に、蛍光抗体法でウィルス抗原を証明することに成功した。これは迅速、適確な診断法として世界最初に開発されたものでありすでに活用されている重要なデータであり、以下の論文がLancet（英国ロンドン）1982年1月2日号５３頁に掲載された。

DETECTION OF DENGUE VIRUS BY

IMMUNOFLUORESCENCE AFTER INTRACEREBRAL

INOCULATION OF MOSQUITOES

In this laboratory dengue viruses are isolated by intrathoracic inoculation of mosquitoes with clinical material obtained from patients with dengue haemorrhagic fever (DHF). Viral antigen is sought by direct fluorescent antibody technique (DFAT) on day 14.

We have now developed a system of intracerebral inoculation of *Toxorhynchites splendens* mosquitoes with dengue-2 prototype virus. The virus could be detected by DFAT as early as day 5. Concurrent intrathoracic inoculation of *T. splendens* with the same prototype virus permits detection of viral antigen on day 10-14. This is the first time that an intracerebral inoculation technique has been described for detection of dengue virus. The technique is as reliable and as specific as the standard intrathoracic method, but it provides for earlier detection of dengue virus, within 5 days.

Earlier work (unpublished) had shown that mononuclear cells are also a good source of dengue antigen, so the cells inoculated were mononuclear ○

cells (buffy coat) obtained by separation on 'Ficollpaque'. 2-4 day old *TX. splendens* mosquitoes were used for mosquito inoculation. The intracerebral inoculation was done through the dorsal part of the head capsule, which lies between the neck and vertex (occiput). The amount of inoculum was 0.17 µl per mosquito. Infected mosquitoes were held at 32°C, relative humidity 80%, and fed on 10% sugar solution. Viral antigen was sought daily by DFAT. On the 4th post-infection day, viral antigen in the form of typical perinuclear fluorescence was detected.

In a preliminary study of blood samples from 68 patients (63 non-shock, 5 shock) with a diagnosis of DHF reached by the intracerebral inoculation method, dengue viral antigen was detected in the head squash preparations (non-shock 13/63, shock 1/5) of the samples on the 5th day. None of the samples negative for dengue viral antigen on the 5th day were positive when examined again on 6th, 7th, and 8th days.

Virus isolation based on an *Aedes pseudoscutellaris* (LSTM-AP-61) cell line had been shown to provide presumptive diagnosis of dengue when cytopathic effect (CPE) was seen as early as 4-6 days after inoculation, but these cultures with CPE need to be tested and confirmed by complement fixation later. With our intracerebral inoculation method, dengue virus can be detected by DFAT from the 5th day after inoculation.

Further tests are now being done to compare the sensitivity of intracerebral inoculation with that of the intrathoracic method from blood samples of DHF.

2. 間接蛍光抗体法による培養細胞（LLCMK$_2$）内デングーウィルス抗原の証明

Dr. Mi Mi Khin

ラングーン子供病院に入院中のデングー熱患児末梢血を蚊の胸部内に接種し、その蚊の頭部磨滅標本（ウィルス抗原陽性）を－８０℃超低温保存をしておき、これを培養細胞LLCMK$_2$株に感染させて、そのウィルス抗原の所在を蛍光抗体法直接法で観察することに成功した。この方法も世界で最初の試みであり極めて重要な成果として高く評価された。

3. 免疫酵素抗体法によるデングーウィルス抗原の証明

U Thet Win

デングー2型ウィルス株をVero 細胞に感染させ、そのウィルス増殖型式をペルオキシダーゼ免疫組織法で検出するのに成功した。この方法だと従来は感染後7～9日も要していたものがわずか3日で証明することが出来、技術的にも非常な進歩を示したものである。

4. デングー熱患者血清の日脳ウィルスに対する中和反応について

Dr.Kyi Kyi Khin

これは大変興味あるかつ重要な研究成果で現在、ビルマのみならず、タイ、バングラデシュ、マレーシアなどでもトピックとなっているテーマである。それは、ビルマでは日本脳炎の流行地であり、多くの患者の出るシャン州、カチン州などでは、デングー熱が非常に少く、逆にデングー熱流行地のラングーンや南部ビルマでは日本脳炎が少い。このたびの研究でその原因の一つであるデングー熱ウィルスに対する患者抗体の中に、日本脳炎ウィルスと中和反応を起す交叉性のあることが明確に証明された。しかし、その逆の日脳患者血清中からはデングー熱ウィルスに対する中和抗体は目下のところ証明されていない。今後の検討を要するところである。

2月19日　午前9時30分～12時30分

免疫学部門研究発表

1. モノクローン抗体（ハイブリドーマ）技術の確立

Dr. Tun Pe

JICAプロジェクトにもとづいて細菌性腸管疾患ならびにアーボウィルス感染症の免疫学的研究を実施する一貫として、特異性のもっとも高い単クローン抗体を得る必要があり、それには近代免疫学のもっとも先端をいくハイブリドーマ手技の確立を試みている。

未だ端緒についたところであるが、1981年にはDr. Tun Pe とU Thet Win 両名がシンガポールで開催されたWHOハイブリドーマ講習会に参加し、またDr. Khin Ohn Lwinは濱島教授の招待で千葉大学で開催されたAMBO（Asian Molecular Biology Organization）ハイブリドーマ講習会に参加。夫々のtraining を受けて帰国し、この開発に取り組んでいる。

2. 蛇毒トキソイドの開発

Dr. Khin Ohn Lwin

前主任Dr. Aung Khin と共に過去2年間にRussell 毒蛇から蛇毒トキソイドの精製を試み、免疫学的分離法にてその純化に成功、現在、ビルマ国産のトキソイド生産にかかり、実地応用にまで到っている。生産はBPI（ビルマ製薬研究所）が行っている。

3. ELISA法による蛇毒検定の研究

Dr. Khin Ohn Lwin

毒蛇咬傷患者末梢血中の蛇毒濃度の迅速測定法を、ビルマ国で開発された抗蛇毒トキソイド抗体を用いてＥＬＩＳＡ（酵素標識免疫血清測定法）による迅速検定を行なうことを確立し、すでに実地に応用している。これは 350 という多数の検体をわずか２分で判定し得るすぐれた方法で、本医学研究局で最近開発し得たものである。

病理学部門

1. デンゲー出血熱の血管凝固の病理

Dr. Than Than（主任）

ビルマにおける感染症で重大な問題点は、感染に伴う血管内の重篤な病変である。とくにデンゲー熱による出血の原因が微細循環系の血管内血球集合、栓塞、免疫複合体による血管障害などが主であり、免疫組織学的検索によってこれらの病因を追求している。

2. ヒト脳性マラリアの血管病理

Dr Than Than

ビルマにおける死因の一位を占めるマラリア脳症の病因について検討中、とくに脳微小血管内のキニンの証明と分布、およびマラリア原虫による直接傷害の過程などを詳細に報告した。

3. 実験マラリアマウスの血液交換

Dr. Maung Maung Oo

この報告は極めてすぐれたかつ、ドラマティックな成果を示したものである。しかもこれが報告者の独創によるものである点、高く評価された成果である。もっとも重篤型を示す熱帯熱マラリアの病原体であるプラスモジウム　バーゲイ（Plasmodium berghei）をマウスに接種すると、３週間以内に重症マラリアで全例死亡する。彼はその接種８日目を選んで、眼球静脈叢を破壊して出来る限り採血し、同時に尾静脈より同系健康マウス血液の輸血を行なった。その結果、全身諸臓器に充満していたマラリア色素は見事に消失し、予想以上の好結果を得たと報告した。これは未だ多くの問題を残してはいるが、今後臨床応用に可能な方法としてその成果が大いに期待された。

２月１９日　午後２時～５時

動物センター

動物センターの発展と供給状況

U Khin Maung Zaw

動物センターは中川博士の極めて適切な御指導と、センター全員の大変な努力によって素晴しい成果が挙っており、すでにビルマ全国からの実験動物の需給に応じている許りでなく、マウスに到っては増産過多のため繁殖制限を行なわなければならない程である。しかし飼糧は未だ手造りであるために非常な労力を必要としており一日も早く自動化機械ペレットの設置が望

まれる。

1980年12月より1982年1月まで14ケ月間に、マウス生産46,429匹、供給された数は33,836匹、ラットは1,093匹産れ、740匹が実験に供され、モルモットは228匹、内72匹が供給、ハムスターは214匹、内104匹が使われ、家兎は71羽出産、55羽が使用された。とくに家兎は、平均体重4kgという、わが国の研究家のものよりも優れており、かつ、新生児マウスも毎日1,000匹以上使用してもなおかつどんどん増えており極めて理想的な運営である。

医学図書館

医学図書館の現状について

Daw Hla Kyi

現在未だ医学図書の利用者は極めて少ないが、他の医学校、病院の医師の研究認識と勉学意欲は次第に高まりつつある。なおこの医学図書館内にあるConference Roomは1981年12月より1982年3月の間に、4種類の国際会議が開催され、次第に活気を帯びてきたことは特筆に値いする。

臨床医学研究センターの報告

1. 乳児下痢症の母乳と人工栄養の影響

   Dr. Daw Che Nwe Oo

2. 急性下痢症児童への経口補液療法の改善

   Dr. Khing Maung U

3. 急性下痢症に対するBecozamycinの効果

   Dr. Khing Maung U

以上

この2日間に及ぶ数多の研究発表は、過去2年間の生物医学研究センター発足以来のものとして、その顕著な進歩は驚く許りの好成果を挙げつつあるものと認めざるを得ない。

また、個々の発表後は日本人専門家側から、とくに団長、吉田教授、中根教授、林助教授より非常に厳しい質問が沢山出て、このエバリュエーション調査団に対する研究発表は内容とその活発な討論共に予想以上の成果の挙っていることを痛感した次第である。

しかし研究レベルは緒についた初段階のものであって、このままで放置したのでは今後の発展は望まれず、以後の延長されることが強く望まれる。

Department of Medical Research

Ministry of Health

Socialist Republic of the Union of Burma

and

The Japanese International Cooperation Agency

Government of Japan

Research on Major Arboviral Diseases, Bacterial Enteric Diseases and the Application of its result for the control of these diseases Project

REPORT

1980 - 1982

F O R E W O R D

The DMR/JICA Project 'Research on Major Arboviral diseases, Bacterial Enteric diseases and the Application of its results for the control of these diseases' was agreed upon and the Record of Discussion signed by the two parties on 10th April 1980. The DMR was given overall responsibility for the Project with the guidance of the Co-ordinating Committee.

This marked a new phase in technical co-operation between the DMR and JICA.

In this Report I have summarized the progress made in the implementation of the Project, and have indicated future possibilities.

Dr. Aung Than Batu
Director-General
10th February 1982.

C O N T E N T S

Annexes

1. Introduction.

The Department of Medical Research (DMR) and the Japanese International Co-operation Agency (JICA) signed the Record of Discussion for the implementation of the Project on April 10, 1980. The term of the Project is for 2 years unless otherwise agreed upon by the two Governments.

The Project aims to conduct research on major arboviral diseases and bacterial enteric diseases with a view eventually to use the results of these studies in the control of these diseases.

The activities under the Project are to be carried but at the Biomedical Research Centre (BRC), which is an integral component of the Department of Medical Research and in the field study areas.

The activities are:-

(a) Research on major arboviral diseases and the application of the result in the control of these diseases

(b) Research on bacterial enteric diseases and the application of the result in the control of these diseases

(c) Further development of laboratory technology and other services.

Research on arboviral diseases and bacterial enteric diseases have been going on at the DMR before 1980 but there were constraints in equipment, supplies and in expertise. Burmese scientists and personnel in the relevant Research Divisions were in a position to immediately start working even in April 1980 on some of the activities planned to be implemented under the Project. Also preparations were made in readiness for research activities to be

carried out when supplies and experts arrive.

Equipment, Supplies and Japanese experts started to arrive in October 1980, and since then activities have taken place at an accelerating pace. Japanese and Burmese scientists and personnel collaborated in defining specific targets, charting approaches and in carrying out the research.

The following sections of this Report summarizes the various activities executed under this Project.

Although this Project is confined to research on Bacterial Enteric Diseases and Arboviral Diseases the nature of scientific research is such that research activities under the present DMR/JICA Project has inevitably interacted with other projects and research activities carried out by DMR. Also, because the BRC is an integral component of DMR, development of technology and facilities under this project has inevitably overflowed and conferred advantages to other DMR Projects. Inorder that a broader perspective may be obtained of the total impact of the DMR/JICA Project, other DMR research activities which have interacted with this Project or have benefited from the technology and facilities developed under this Project have been mentioned in a sperate section of the Report.

A vitally important but intangible achievement is the cross-fertilization of ideas and stimulation which have further taken place in DMR as a consequence of the implementation of the DMR/JICA Project. Such an important result cannot be delineated in a Report of this nature but should be implicit in the activities mentioned.

2. Bacterial Enteric Diseases Research.

2.1 Research on certain epidemiological, nutritional and community-based theraputic aspects of diarrhoea have been in progress at DMR before 1980, but they were deficient in the identification of the responsible bacterial pathogens.

From the beginning of the DMR/JICA Project in April 1980 it was clear that identification of the etiological agents responsible for acute diarrhoea in Burma was the most important objective of the Bacterial Enteric Diseases Research under the DMR/JICA Project and that epidemiology and mode of transmission was the next objective. A third objective, viz. study of plasmid mediated resistance and virulence factors was formulated after arrival of the Japanese Expert late in 1980. (Annex 2)

The first phase of the Research was to establish the most appropriate and advanced methods for the identifications of bacterial pathogens (and viral pathogens) responsible for acute diarrhoea. Very creditably this was accomplished within a few months after arrival of supplies and the Japanese Expert. New and better methods for the identification of bacterial pathogens especially Enterotoxogenic E.coli are being developed in research centres throughout the world and the Bacteriology and Virology Research Divisions of DMR are testing and adopting these methods, such as the ELISA and BIKEN.

The second phase of the Research was to identify the major etiological agents responsible for diarrhoea in an urban community in Burma, and to determine their relative importance. A hospital and community-based study of the etiological agents responsible for acute diarrhoea in urban children was carried out during the cold season of 1980 and Monsoon season of 1981.

North Okkalapa township in Rangoon with total population of 1846000 and 1545 of under-five children was chosen as the field area. The pattern of antibiotic resistance of the bacterial pathogens as well as certain aspects of epidemiology were also studied. A full scientific report is contained in the 'Research Findings' attached to this Report.

In the third phase the major etiological agents in a rural community would be studied and this is planned for the Monsoon of 1982.

2.2 An investigation of the mode of transmission of ETEC was planned to be carried out simultaneously with the study of etiological agents but this was not possible principally due to inability to serotype E.coli in DMR as well as in Japan.

Arrangements have been made to serotype the isolated E.coli in a WHO Reference Laboratory but this capability would have to be established in DMR if transmission studies are to be effectively carried out as planned. More rapid methods of identifying ST and LT strains of ETEC would also need to be developed and set up at DMR.

2.3 Methods for the study of plasmid in bacteria are now being set up in Bacteriology Research Division of DMR but considerable time, expertise and material will need to be invested before this approach to Bacterial Enteric Diseases Research could be actually pursued.

2.4 Gas chromatographic method for identification of certain types of bacteria has been introduced by the Japanese Expert but needs further development.

2.5 The identification of the major etiological agents, their relative importance and the antibiotic sensitivity pattern will have direct application in the control of acute diarrhoea in children. This is the leading cause of mortality and morbidity among children in Burma.

3. Arboviral Diseases Research.

3.1 Research on Arboviral Diseases has been conducted in the Virology and Entomology Research Divisions of DMR even before 1980. The specific research objectives of the Arboviral Diseases Research to be carried out under the DMR/JICA Project were first developed in April 1980 but took definite form only when the Japanese Expert in Virology arrived at the end of 1980. These were further amplified and modified in 1981. (Annex 2). Dengue Haemorrhagic Fever (DHF) is the foremost arboviral disease in Burma and in the context of the research already done on DHF by DMR and other organizations in Burma and in view of the current state of knowledge and outstanding problems pertaining to this disease it was decided that the first objective of the Arboviral Diseases Research under the Project would be to identify biological markers associated with virulence of the dengue virus responsible for DHF and Dengue Shock Syndrome (DSS).

3.2 Identification of biological markers associated with virus virulence in DHF and DSS is a fundamental research problem which calls for basic research in Virology and for establishment of certain virological techniques hitherto not yet available at the Virology Research Division of DMR.

There was considerable delay in starting this research due to the slow mobilization of the materials and expertise required. A beginning has been made in the second year but considerably more inputs in terms of expertise and supplies will be required before this research gains momentum.

3.3 The second objective of Arboviral Diseases Research under this Project was to study further the cross protection between Dengue virus and Japanese Encephalitis virus

infections. It was thought that opportunities might present itself in Burma for further investigation and clarification of this scientifically and practically important relationship between Dengue and J.E infections.

A laboratory investigation has been completed which confirms previous reports by others. A full scientific report of this investigation is given in the 'Research Findings' attached to this Report. Further investigations are planned.

3.4 The third but low priority objective of Arboviral Diseases Research under this Project was to develop rapid, sensitive methods for the identification of dengue virus and arboviruses.

Good and early results have been obtained in this area. A modification of the mosquitoe inoculation of dengue virus isolation has been introduced. This new method is the intracerebral inoculation into mosquitoes of the mononuclear cells separated from patients' blood and has considerably shortened the time required for identification of the dengue virus by immunofluorescence. A full scientific report is given in the 'Research Findings' attached to this Report.

Another new method of rapid identification developed (whilst one of the trainees was in Japan at the Virology Department, Kansai University) was a modification of the immuno-peroxidase method of staining dengue plaques, which has shortened considerably the time required for identification of dengue virus. A full scientific report is given in the 'Research Findings' attached to this Report.

3.5 Research on the bacterial enteric diseases and the major etiological agents responsible for acute diarrhoea

soon indicated that viral pathogens are an important cause of diarrhoea and that to neglect this aspect of research on acute diarrhoea would reduce the value and impact of research on Bacterial Enteric Diseases. It was decided that methods for the identification of the most important viral pathogen, viz. Rotavirus should be established in the Virology Research Division. Identification of Rotavirus by electron-microscopy is being done in Virology Research Division but needs verification. More advanced techniques such as the ELISA also need to be established.

4. Development of Immunology.

4.1 Since immunological concepts and methods are basic to the study of bacterial enteric diseases and arboviral diseases development of immunological knowledge and technology was accepted as a component of the Project.

4.2 Hybridoma technology and production of mononuclear antibodies was considered to be a recent development which would be of extreme importance for furthering the aims and objectives of the DMR/JICA Project. It is the type of technological advance which would enable DMR to leapfrog to the vanguard in certain fields of research. There was also a reasonable possibility that this technology could be established in DMR under the DMR/JICA Project and it was decided that the prime objective of development in Immunological knowledge and technology under this Project would be to establish the Hybridoma technology in the Immunology Research Division of DMR.

Accordingly a plan for training and procurement of supplies has been drawn up and is being implemented. (Annex 2). Already, three scientists have attended Workshops on Hybridoma in Japan and Singapore. Considerable time and development of expertise would be needed to achieve the above aim.

5. Development of Pathology.

5.1 The development of pathological knowledge and techniques, being an essential requisite for productive research on bacterial enteric diseases and arboviral diseases, was accepted as a component of the Project.

The Pathology Research Division of DMR was re-organized, the principal change being that the Electron Microscope Laboratory was now placed under the Pathology Research Division. Areas of development more relevant to the future research aims of the DMR and the DMR/JICA Project were identified. (Annex 2).

5.2 One important aim of pathological expertise was to develope the capability to undertake studies on bleeding defects and disturbances of the microcirculation in Dengue Haemorrhagic Fever. This would also enable the Division to study such disorders in other important infective diseases in Burma such as Malaria and Snake-bite.

Routine basic laboratory methods for the study of bleeding disorders and rheology were set up and histological techniques were improved. Short small group training courses in immunopathological concepts and techniques and in electron microscopy were conducted by the Japanese Experts. (see also sections 9 and 10). Measures were taken to extend the usage of the electron microscope and preparations were made to set up the new transmission electron microscope in the Pathology Research Division, Biomedical Research Centre building.

6. Development of Laboratory Animal Technology and Facilities.

An essential support facility for effective research in Bacterial Enteric Diseases and Arboviral Diseases is the development of Laboratory Animal Technology and facilities and this was vigorously implemented.

New stock of laboratory animals were received from JICA. The Japanese Expert who arrived near the end of 1980 and the Burmese counterpart very quickly organized the services so that high quality animals were already available by the time Bacterial Enteric Diseases Research program started.

The Laboratory Animal Services are now fully operational. Satisfactory work is being done both in the breeding and experimental sections.

It was found that manual food pelleting could not cope with the demand and a Pelleting machine will arrive before the end of February 1982.

Disease control work is being carried out with the co-operation of the Bacteriology and other relevant Research Divisions of DMR. This aspect of work would need to be strengthened within the Laboratory Animal Services.

The breeding and supply record of the Laboratory Animal Services are shown in the following tables.

LABORATORY ANIMAL SERVICES DIVISION

(From 1980 December to 1982 January)

No	Animal Species	Production	Supplied
1.	Mouse	46,429	33,836
2.	Rat	1,093	740
3.	Guinea Pig	228	72
4.	Hamster	214	104
5.	Rabbit	71	55

ANIMAL SUPPLIED CHART

(From 1981 February to 1982 January)

S/No	Divisions	Mouse	Rat	Hamster	Guinea pig	Rabbit	Sheep	Goat	Fowl	Goose	Dog	Monkey
1.	Bio-chemistry	72	220	51	8	1	-	-	-	-	-	-
2.	Bacteriology	24937	5	-	34	12	-	-	5	-	-	-
3.	Immunology	550	25	-	14	5	2	-	-	-	-	-
4.	Nu-medicine	121	18	-	-	-	-	2	-	-	-	-
5.	Entomology	163	-	-	-	-	-	-	6	-	-	-
6.	Parasitology	1106	81	-	-	3	-	-	-	-	-	-
7.	Pharmacology	105	25	-	8	5	-	-	-	-	-	-
8.	Pathology	2838	2	53	8	19	-	-	-	-	-	-
9.	Virology	5234	-	-	-	10	-	2	-	5	-	-
10.	Burma Pharmaceutical Ind.	1350	6	-	-	-	-	-	-	-	-	-
11.	National Health Lab.	650	-	-	-	-	-	-	-	-	-	-
12.	Children Hospital	400	-	-	-	-	-	-	-	-	-	-
13.	Medical Institutions	20	358	-	-	-	-	-	-	-	-	-
14.	Arts & Science University	220	-	-	-	-	-	-	-	-	-	-
	TOTAL	37766	740	104	72	55	2	4	11	5	-	-

7. Development of Instrumentation Technology and Facilities.

Development of the capability to maintain, and repair the equipment and instruments for the Project was recognized as an important support service for the DMR/JICA Research Project.

The Project assisted the development of instrumentation technology by training an Engineer in Japan. Government has also strongly reinforced the scientific and technical personnel in the Instrumentation Division so that better and more regular maintainence and repair services are now possible (see also section 14). This Division will continue to need steady support of spare-parts for the instruments.

8. Development of Library Facilities.

Provision of adequate Library facilities is essential to research in any subject.

This Project could not give high priority to Library development. However, the Government budget has continued to provide fairly satisfactory supply of journals and has also expanded the staff considerably. (see section 14).

Linkages should be made with the Libraries of some Universities in Japan for exchange and computer search facilities.

9. Training.

Training is one of the components of the DMR/JICA Project and consisted of the following:

(1) Training of Burmese scientists and technicians in Japan.

(2) Training of Burmese scientists and technicians in DMR by Japanese Experts and Burmese counterparts.

(1) Training in Japan.

Altogether 3 scientists - one in Pathology, one in Virology and one in Instrumentation and 2 senior technicians - one in Virology and one in Entomalogy are being trained at various institutions in Japan. One other technician in Bacteriology is also scheduled.

These trainees were chosen after consultation between Japanese scientists and Burmese scientists based on the needs of the research and development program.

Placement in institutions in Japan is primarily the responsibility of the Japanese authorities, taking into consideration the type of training requirement. Great care would need to be taken to see that the training received matches the requirements. This components of the Project is proceeding satisfactorily and is of great help to the development of research and expertise in DMR.

In addition to the trainees mentioned above, one Engineer and one Laboratory Animal technician were trained by JICA under the regular country program (but not under this Project) in anticipation of the implementation of the present DMR/JICA Project. The two trainees have greatly accelerated development of facilities in DMR and made possible the rapid progress in research.

(2) Training in Burma.

In addition to the exchange of knowledge and skills which takes place between Japanese and Burmese scientists and the on-the-job training which accompanies the collaborative conduct of research work, Japanese scientists gave short small-group training in various subjects so as to impart knowledge to a wider audience.

Small-group courses were conducted as follows:-

(a) Immune-pathology by Professor Y. Hamashima
(b) Electron microscopy by Professor K. Nakana
(c) Gas chromatography by Professor Y. Kanamasa.

10. Japanese Experts.

This component of the Project provides Japanese Experts to come to DMR to help and advise with the execution of research and development of technology.

Japanese Experts in Bacteriology, Virology, Pathoimmunology, Animal technology, Electron-microscopy have come to DMR under this Project according to the needs of the Project as detailed in the Work Programs. (see Annex 2)

The role of the Japanese Experts have been mentioned in the relevant sections of the Project. They have generally contributed in a valuable way toward progress of research and development of the necessary technology.

11. Supplies and Equipment.

This vital component of the Project was vigorously implemented and involved extensive and repeated consultation between Japanese and Burmese scientists.

Lists of requirement were drawn-up according to the needs of the Project. Burmese scientists were not in a position to know the availability of the Japanese products and JICA undertook the ordering of supplies on the advice of an Advisory groups of Japanese scientists in Japan.

Supplies started to arrive in late 1980 and consisted of the following categories:-

(a) Equipment

(b) Chemical reagent, laboratory supplies and glassware

(c) Spare-parts for instruments and equipment.

Supplies and equipment for the year 1980-1981 have all arrived. A major item is a CO_2 incubator.

Supplies and equipment for the year 1981-1982 have been requested but has not yet started to arrive. Major item for this year are:- Pelleting machine and a Electron microscope (transmission).

JICA and the Japanese scientists have endeavoured to supply the requirements to the fullest extent within the limits of the funds available. Because of the nature of research programming and the consultations involved, there were a number of instances of dislocation between

arrival of supplies and time of requirement. Better information exchange and streamlining of procedure is necessary.

12. Other DMR Research Activities which interact with DMR/JICA Project.

The Department of Medical Research under-takes a wide range of research activities in its 15 Research Divisions, in its Clinical Research Units attached to hospitals, and through collaborative arrangements with other institutions in the country.

Although not concerned with Major Arboviral Diseases or Bacterial Enteric Diseases and therefore not directly under the DMR/JICA Project, some of these other research activities interact with those under this Project since there can be no clear cut boundaries in scientific research. Also some research activities, although not concerned with arboviral diseases or bacterial enteric diseases, are carried out in Divisions strengthened by this Project and has benefited by the development in technology.

Some of these other DMR research activities which interact with the research and developmental activities of this DMR/JICA Project are summarized below, inorder that a proper perspective and assessment of the Project may be made.

1. Pathology

(a) Measurement of F.D.P and clotting defects in human cerebral malaria

(b) Histopathological studies on human cerebral malaria

(c) Effect of exchange transfusion on histopathological lesions in P.berghei malaria in rats.

This is an innovative approach to the treatment of cerebral malaria and the pathological basis for this approach is being investigated in experimental animals with a view to eventual application in

man. The results are promising and this study will have to be extended.

2. Immunology

(a) Development and testing of Russells Viper Venom Toxiod.

Preliminary work in experimental animals was completed in the previous years. During 1980-81 Human volunteers were tested with a toxiod of refined viper venom developed in DMR. Satisfactorily high levels of antibody response was obtained lasting up to 6 months. There were no severe side effects. This is the first time a Viper Venom Toxiod has been developed and tested in human subjects.

(b) Development of ELISA for viper venom and observation of the kinetics of viper envenomation in animals.

3. Bacteriology

Monitoring of Drug-resistant M.Leprae using mouse-foot-pad method. This study has shown that drug-resistance to Dapsone has developed in Burma. Further studies on factors influencing drug-resistance are being pursued.

4. Virology

(a) Research on transovarial transmission of Dengue Virus in nature which was begun earlier was continued and completed in 1980. This work has an important and direct bearing on arboviral Research activities being whether under DMI/JICA Project.

5. Nutrition and Clinical Research

(a) Study of breast-feeding and weaning practices in urban working mothers.

This study has a direct relationship with bacterial enteric diseases research since breast-feeding and weaning practice will influence the prevalence and distribution of acute diarrhoea in children.

(b) The efficacy and acceptability of oral rehydration therapy administered by village mothers.

This study has shown that oral rehydration therapy given by village mother is effective in preventing dehydration but that the long term nutritional improvement claimed by others is not confirmed.

It has direct bearing on research in bacterial enteric diseases.

(c) Clinical trial of Becozamycin, tetracycline and placebo in acute diarrhoea.

6. Epidemiology

(a) Development of epidemiological model for Ascaris infection in the community.

Development of this epidemiological model for ascaris infection will enable a similar model to be developed for bacterial enteric diseases (acute diarrhoea) and facilitate the assessment of impact of intervention programs.

13. Academic activities.

The DMR conducts and participates in a variety of academic activities which are:-

(a) Attendance at Meetings, seminars, workshops - both national and international.
(b) Scientific talks and discussions in DMR.
(c) Supervision of research of post-graduate students from Institutes of Medicine and Arts and Science Universities for the degree of M.Med.Sc and M.Sc.

At present there are 5 Master students in DMR working on topics relevant to the Project or in Divisions strengthened by this Project.

Some of the various academic activities are listed in Annex 5.

14. Staff development, deployment and other DMR inputs to the Project.

(a) Staff deployment

The following staff are employed in Research Divisions directly concerned with the Research Project.

Virology Research Division	- 5 scientists
	- 9 technicians
Bacteriology Research Division	- 4 scientists
	9 technicians
Pathology Research Division	- 3 scientists
	5 technicians
Immunology Research Division	- 4 scientists
	5 technicians
Entomology Research Division	- 2 scientists
	5 technicians

In addition, staff from Clinical Research, Epidemiology and Medical Statistics Division are deployed on a large scale during field studies on Bacterial Enteric Diseases and collaboration is obtained from hospital and other health staff in the field-area and from the community leaders.

(b) Staff development

Altogether 133 new staff posts were created in DMR during 1980-82 comprising 29 scientists, 43 technicians, 61 admisnistrative staffs including lower echelon workers. 12 scientists, 26 technicians are for Research Divisions concerned with the DMR/JICA Project or for Technical support services.

(c) Others

Motor vehicles of DMR are utilized extensively during the field studies for this Project.

The Divisions concerned continue to draw from DMR Central Stores for part of their requirements.

ANNEX 1

MASTER PLAN OF THE PROJECT

1. Objective

The Project aims to conduct Research on major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases.

2. Implementation

The Department of Medical Research has overall responsibilities for the Project with the guidance of the Coordinating Committee. The Biomedical Research Centre which is an integrated functional component of the said Department is the executing organ for the achievement of the above mentioned objective.

3. Activities under the Project

Activities under the Project will be carried out at the Biomedical Research Centre premisesincluding the pilot area:

Activities will include the following:

(a) Research on major arbo-viral diseases and the application of its achievement for their control.

(b) Research on major bacterial enteric diseases and the application of its achievement for their control.

(c) To further develop technology of laboratory and other services.

Annex - 2.

Arboviral Diseases Research

Virology Research Division and Entomology Research Division

Work plan for 1980 - 1981.

The Virology Research Division of the Department of Medical Research will collaborate with the Japanese Expert under the JICA Research Project for the year 1980 - 81.

Work programme will be centered on elucidating problems in relation to arbovirus infections which are of public health concern. The ultimate aim will be directed towards control of diseases so as to benefit the community.

Proposed Programme areas.

1. Study of the virulence of dengue viruses by looking for genetic markers in viruses of different serotypes and different grades of illness.
2. Interrelationship of cross protectior to JE infections in a highly dengue endemic area.
3. If possible the Virology Research Division wishes to establish (a) the technology of ELISA and (b) production of monoclonal antibodies.

Proposed work programme for Japanese expert (2 months in 1980-81)

1. Planning of the research programme on arboviral infections.
2. Scientific planning of the programme in arboviral infection.

1. To study the virulence of dengue viruses by looking for genetic markers in viruses of different serotypes and different grades of illness.

   1.1. Serotyping of the dengue isolates obtained from clinical materials by the complement fixation test.

   1.2. Preparation of pools of low passage virus in mosquitoes and cell cultures.

1.3. Titration of the virus pools of low passage virus in mosquitoes and cell cultures.

1.4. Genetic purification by plaque picking in cell cultures.

1.5. Temperature sensitivity patterns of dengue viruses.

1.6. Sensitivity to protease.

1.7. Vector competence, transmission, and virulence for infant mice.

1.8. Cytopathic effect on mammalian and arthorpod cell cultures.

1.9. Morphological observation by EM the development of dengue viruses in mammalian and arthropod cells.

It is desired that: -

Item - 1.4, 1.5, and 1.6 will be eludicated during the presence of the expert.

Reasons for the need of research on virulence.

Background information.

1. Classical dengue infections have been known to occur as early as the 18th century (Greece 1928 - clinically diagnosed).

However since the last three decades severe haemorrhagic disease have been known to be caused by dengue viruses of four serotypes, and have become a major public health problem in west regions of South East Asia and Western Pacific Regions. Biological behavior of viruses due to transfer from its original hosts to the vectors or vice versa may result with spontaneous mutation. Such variations may either be in the form of emergence of strains of viruses which are attenuated and of low virulence, or in the extreme form, there may be a dramatic shift from low virulence to the virulent forms, thereby exhibiting a change in the pathogenetic mechanism from either inapparent or mild to the severe form of infection.

2. Laboratory finding.

A hypothesis had been formulated that the severe form of dengue DF/DSS is a resultant of multiple infection of dengue endemic region, and the severe form is due to immune complexes having a direct action of the vascular system thus resulting with leakage of the plasma volume and shock. This would undoubtedly mean that DSS would be encountered only in the secondarily infected patients. However our findings together with a few others have found that DSS also occurs in the patients exhibiting a primary antibody response to dengue (10-11% of the DSS patients have primary antibody response). It thus initiates us to reason whether there are emergence of some virulent form within a serotype, or are certain serotypes more virulent then others as in the case of poliomyelitis and influenza viruses.

Reason for the need of studies on the interrelationship of cross protection to JE infections in a highly dengue endemic area.

Background information.

Japanese encephilitis (JE) is an infection of the central nervous system caused by a group B arbovirus and transmitted to man through mosquito (Culicines). It has been occuring in S.E.Asia for a considerable time. Although epidemics were known to occur in Japan and Korea small outbreaks have been known to occur since 1955 in various regions of S.E. Asia (Thailand, Burma, India & Bangladesh).

Laboratory finding.

J.E. infections are known to occur as occasional small epidemics in the North-eastern region of Burma (Dr. Than Swe, National Health Laboratory). A small study was carried out to see the prevalence of JE NT antibodies among the porcine and human population. Although 90% of the pigs had NT antibodies to JE a low incidence was encountered in the human population who had HI antibodies to dengue (90%).
It thus initiated to elucidate whether the human population due to the presence of dengue antibodies were protected against JE infections.

Arboviral Disease Research

Virology and Entomology Research Divisions

Work plan for 1981 - 1982

A. Work objectives (1981-82)

1. Study of virulence as a determinant of disease severity in DHF.

1.1. Will collect and bank dengue virus isolates from clinical material.

1.2. Will set up following test systems of biological markers of virulence.

(a) ---- infectivity titration in mammalian cell culture and mosquito system.

(b) ---- plaque assay

(c) ==== purity picking of selected plaque clones

(d) ---- temperature sensitivity testing

(e) ---- sensitivity to protease

(f) ---- purification by protamine sulphate ethanol treatment

(g) ---- infectivity assay in mosquitoes, mammalian, and new born mice systems

(determine virulence in the mouse system).

1.3. Will test some isolates from stage (a) to (d).

2. Cross protection between JE and Dengue virus infection.

Phase 1 hospital based study:

2.1. Will collect acute and convalescent serum from hospital patients with DHF and to obtain at least 50 sera with dengue neutralizing antibodies.

2.2. Set up plaque reduction neutralization test for both dengue and JE.

2.3. Test some of the collected sera with the above system. (if possible - all).

Phase 11 community based study:

Will plan and prepare and if possible collect sera of the community for subsequent test.

3. Development of rapid sensitive and specific laboratory diagnosis for arboviral infection by ELISA.

Plan and prepare for development of ELISA test for detection of dengue antigen during the acute stage of infection.

4. Rotavirus:

Will collaborate with Immunology Research Division (Electron Micro Lab), in detecting Rotavirus from stool specimen from diarrhoea surveys.

B. Supplies and equipments:

1. All major equipments for virulence and cross protection studies are available.

2. Reagents, chemicals and stationary have been requested in 1980-1981 and 1981-1982.

3. It is essential to obtain the following for the virulence study:-
   (a) Mouse immune ascitic fluid for dengue 1,2,3,4 & JE virus
   (b) Haemolysin
   (c) Complement
   (d) Freund's adjuvant complete & incomplete.
   (e) High titered prototype smbr of Dengue 1,2,3,4 & JE virus
   (f) Sucrose acetone extracted smbr HA antigen of Dengue 1,2,3,4 & JE.

4. It is essential to obtain the following for the cross protection study:-
   (a) Tissue culture antigen of Dengue 1,2,3,4 & JE for PRNT. (to test 200 to 300 sera)
   (b) Monkey immune sera of Dengue 1,2,3,4 & for PRNT (to test 200 to 300 sera), if monkey immune sera not available other animal hyperimmune sera will suffice.
   (c) Prototype of dengue 1,2,3,4 & JE virus for preparation of tissue culture antigen. (tissue culture adapted in BHK Vero or LLCMK$_2$).

5. Japanese Expert:

5.1. An expert who will be able to set up test system for biological markers for virulence as shown in the study design.

5.2. The Japanese expert should also be able to help set up the methods of preparation of essential biological reagents necessary for research projects and which are not available commercially from external sources.

Reagents are:-

- Dengue mouse immune ascitic fluids to Dengue type 1,2,3,4 & JE.
- Monkey hyperimmune sera for PRNT for Dengue type 1,2,3,4 & JE.
- Tissue culture antigens for the PRNT (Dengue type 1,2,3,4 & JE).
- Tissue culture haemagglutinating antigens for Dengue 1,2,3,4 & JE.

5.3. Japanese expert should plan and advise the scientific feasibility of virulence and cross protection projects.

5.4. For the above requirements it is proposed that at least one Japanese expert should stay for a period of 4-6 months and preferably this expert should be able to do items No. 1 and 2.

5.5. One high level expert who is prominent in the field of arboviruses research including dengue, should be able to stay for about 1-2 months to plan and advise on the scientific feasibility, and if possible help set up some of the test system required.

Burmese Trainee:

1. It is tentatively proposed that a researcher (Virology) will be trained in Japan for 1 year, under 1981-82 fellowship. Formal proposal will be sent through proper channels.

2. Type of training:-

(a) The proposed fellow will be a doctor with Post-graduate Diploma in Bacteriology, and has experience in Virological techniques.

(b) Training will be needed in General Virology techniques, and the special area of interest will be (a) Viruses causing diarrhoea (of which rotavirus is the most important), and (b) including also other Enteroviruses.

The training should include methods to identify viruses as causative agents of acute diarrhoea using standard virological techniques, including the electron microscope as well as other methods of identification, which are likely to be developed later, such as ELISA.
On return the trainee should be able to conduct further independent research on viruses of importance as a cause of diarrhoea and on Enteroviruses.

ARBOVIRAL DISEASE RESEARCH PROGRAMME

1. Study of virulence as a determinant of disease severity in Dengue Haemorrhagic fever

1.1 Scientific Justification

Pathogenesis of the severe form of dengue namely dengue shock syndrome has not yet been resolved to this day. Epidemiological evidence have shown that strains & sero-type of dengue virus(es) may be the key factor in determining the magnitude of an epidemic, as well as the degree of desease severity. In Burma dengue 2 & 3 serotypes were frequently encountered from both the severe D.S.S. and relatively mild nonshock DHF patients.

However it was of much interest to recover more isolates of dengue type 2, from D.S.S patients.

Biological behaviour of viruses due to transfer from its original host to the vector or vice versa may result with spontaneous mutation. Such variations may either be in the form of emergence of strains of viruses, which are attenuated and of low virulence or in the extreme there may be a dramatic shift from low virulence to the highly virulent forms, thereby exhibiting a spectrum of severity. A hypothesis has been formulated that the severe form of DHF/DSS is a resultant of multiple sequential infections of dengue in a highly dengue endemic region and the severe form is due to immune complexes having a direct action on the vascular system, thus resulting with a leakage of plasma and shock. This would undoubtedly mean that D.S.S would be encountered only in the secondary infected patients. However our findings together with a few others have found that D.S.S also occurs in the patients exhibiting a primary antibody response to dengue (10% - 11%. of the D.S.S patients have primary antibody response) i.e., in those who do not have sequential infection but have had of a single infection. This is a strong evidence that -

sequential and multiple infection is not necessarily a determinant of severe diseases. Thus the possibility that, like in other microbial infections, virulence of the virus as the major determinant of severe or mild disease will have to be again considered and investigated.

1.2 Design of study

1.2.1 Biological markers which in other virus infections are known to be indicators of virulence will be studied in dengue viruses recovered from (a) patients with severe disease (DSS, DHF Grade 3 & 4) and (b) patients with mild dengue infection (Grade 1 & 2 DHF).

1.2.2 It will be observed whether there are differences in the pattern and frequency of biological markers (usually associated with virulence) between viruses of each serotype recovered from severe and mild form of diseases. (e.g., dengue type 1 from severe diseases versus dengue type 1 from mild disease).

1.2.3 Detection of such a difference may indicate that virulence is a major determinant of disease severity. That is, its biological markers which in other viruses have been associated with disease severity are also observed to be more frequently associated in severe dengue, then it may be inferred that virulence is a positive major determinant in dengue.
This inference will be strengthened if such differences in frequency of biological markers are detected between viruses from severe diseases with primary antibody response and mild diseases with primary antibody response.

1.2.4 It is obvious that as in other diseases caused by other microorganisms, even if virulence is a major determinant of disease severity, not all patients infected with virulent viruses will suffer from severe disease - which then would be the functions of dose and host factors. Therefore the study of biological markers will have to be

done on a sufficent number of samples of isolates. At least 25-50 isolates from severe, and 25-50 from mild disease for each dengue serotype will have to be tested.

1.2.5 Possible duration of research 2-3 years.

1.2.6 If no differences in the pattern and frequency of occurence of biological markers are detected, then the possible conclusion are: -

(a) There is no evidence that virulence is an important determinant, and that sequential infection may be the major determinant.

(b) The biological markers used are not appropriate for the detection of properties associated with virulence in dengue, and other markers may still reveal difference in virulence.

At this stage a further decision will have to be made as to whether this line of investigation should be further pursued or dropped.

1.2.7 Steps in the investigation of virulence:

(a) Isolation of Dengue viruses from patients with severe and mild disease.
(b) Plaque assay
(c) Cloning and purification
(d) Infectivity assay - (1) MID 50 in mosquitoe
(2) TCID:50 in cell culture
(3) Suckling mice assay
(e) Temperature sensitivity tests
(f) Chemical sensitivity tests
(g) Suckling mouse

1.2.3 (Possible analysis of results)

Biological markers	Frequency in severe disease				Frequency in mild disease			
	Dn-1	Dn-2	Dn-3	Dn-4	Dn-1	Dn-2	Dn-3	Dn-4
A. Plaque size								
B. Temperature sensitivity								
C. Sensitivity to protease								
D. Cytopathic effect on mammalian cell system								
E. Virulence in newborn mice								
F. Viremia in monkeys								

FLOW CHART OF THE STUDY DESIGN

PHASE 1.

SUBJECTS: - Clinical patients with varying degree of disease severity i.e.,
Non shock (Grade 1 & 2)
Shock (Grade 3 & 4)

↓

SPECIMEN - Serum collected during the acute phase of illness (up to 5 days)

↓

ISOLATION - Undiluted serum inoculated intrathoracically into Toxorhynchites, incubated at 30°C with RH of 70-80% for 14 days.

↓

DETECTION OF DENGUE - Check mosquitoes head squash by FA using antidengue conjugate.

↓

SEROTYPE OF DENGUE DETERMINED - Complement fixation test using infected mosquito as the source of antigen 2½ units of complement & dengue 1,2,3,4 & JE immune mouse ascitic fluid

↓

STOCK VIRUS PREPARATION - Preparation of low passage virus in mosquitoes, tissue culture (vero, LLCMK$_2$, BHK 21).

↓

INFECTIVITY ASSAY - Infectivity titration of low passage virus in mosquitoes and mammalian cell culture system.

Since ELISA technology has been proven to be able to detect either the antibody or antigen in a relatively short time, an attempt will be directed in trying to develop ELISA method to detect the antigen in the acute phase serum of dengue/dengue haemorrhagic fever patients.

2. Cross protection between Japanese encephalitis infection

2.1 Scientific justification

Occurence of JE epidemics have been reported in the neighbouring countries of Burma (India, Bangladesh and Thailand). In Rangoon our studies have revealed that JE neutralizing antibodies are present in 90% of the pig population. Among the human population although there is a high incidence of dengue HI antibodies of varying degrees, the percentage of the presence of JE neutralizing antibody was relatively low. Although every situation for JE outbreak prevails fortunately this has not occured. Many workers have given evidence that in experimental animals previous infections with one or more types of dengue, have produced significant resistance to the subsequent infection with JE and closely related Group B arboviruses.

However, epidemiological evidence of such protection against JE by previous infection with Dengue in human populations is still tentative and needs further investigation. There are no laboratory based studies of such a protective effect in human subjects. Because of the importance of the problem to Burma and because of the unique opportunity provided the DTR proposes to study this problem of cross-protection against JE by previous Dengue infection in human population.

2.2 Design of study

Phase 1 Hospital based study

Hospital based study to demonstrate - in sera of patients with Dengue Haemorrhagic fever who have not experiened previous JE infection - neutralizing ability against J.E virus.

Seraof patient with DHF will be tested for the presence of neutralizing antibodies by the PRNT. Previous JE infection will be excluded by pattern of JE antibody response.

Phase II Community study

Community study comparing the JE neutralizing ability of sera from subjects with or without Dengue neutralizing capability.

3. Development of rapid, sensitive and specific laboratory diagnosis for arboviral infections by ELISA

3.1 Scientific justification

Rapid diagnostic service is of much value in aiding the clinician and epidemiologist to carry out prompt effective measures.

Bacterial Enteric Diseases Research Programme

Bacteriology Research Division

(Work plan for 1980 - 1981)

Objective:

1. To identify the major etiological agents responsible for acute diarrhoea in Burma. The emphasis will be on acute diarrhoea endemic among infants and young children in the community.
2. To describe the epidemiology of the major etiological agents including the mode of acquisition of infection.
3. To determine the antibiotic (chemotherapeutic) sensitivity pattern of these major etiological agents (E.coli, Shigella, Salmonella) when this is not yet sufficiently known and to determine the efficacy of various antibiotics (chemotherapeutic agents) in infected subjects.
4. Development of methods of study of colonizing factors (pili) with a view to determining their importance in infection with the major etiological agents in Burma.
5. Development of bacterial genetics studies with a view to helping identify important pathogenic strains of disease causing bacteria in Burma.

1. Identification of major etiological agents of acute diarrhoea

1.1 Development of the capability to identify the following

a) Escherichia coli
b) Salmonella (including typhi)
c) Shigella
d) Vibrio cholerae
e) V parahemolyticus
f) Yersinia enterocolitis
g) Campylobacter jejuni

1.2 With respect to E.coli, development of the capability to identify

a) Enterotoxigenic E.coli (ETEC), stable toxin (ST), labile toxin (LT)

b) Enteropathogenic E.coli (EPEC)

c) Enteroinvasive E.coli (EIEC)

by techniques appropriate to the aims and the available resources.

1.3 With respect to Rotavirus - development of the capability to identify Rotavirus by techniques suitable to the aims and available resources.

1.4 To determine the serotypes and biotypes prevalent in the general population and in the patients with acute diarrhoea in the community.

2. The epidemiology of acute diarrhoea:

2.1 To describe the epidemiology of acute diarrhoea - stratified into:-

A). a) age
b) sex
c) socio-economic groups
d) urban/rural residence
e) seasonality
f) geographical area

B). Clustering in time and space of acute diarrhoea due to each major etiological agents.

C). Relationship to breast feeding, food, water-supply

WORK PROGRAMME

1. Establishment of methods for the identification of the major etiological agents.

1.1 Establishment of capability to handle sufficient number of samples for preliminary screening and storage for definitive identification.

1.2 Establishment of tests for identification of:-

EPEC

ETEC - ST, LT

EIEC

1.3 Establishment of test for Rotavirus

ELISA

TRAINING:

Bacterial Enteric Diseases Research

Bacteriology Research Division

Work plan for 1981 - 82

1. The major etiological agent (bacterial, viral, protozoal) responsible for acute diarrhoea in children ($\leq$ 5 years) will be identified and their incidence among children with diarrhoea in one urban and one rural community (pilot area) at least during the peak monsoon season and if possible during 3 seasons of the year will be investigated.

2. Study of the mode of transmission will be confined to E.coli which has been chosen because this is the organism for which there are large gaps in knowledge regarding mode of transmission in the community. For 1980-81 a longitudinal study will be undertaken on a conhort of a sufficient number of families in an urban area and the acquistion of E.coli (ETEC, EPEC) bacteria will be investigated in all members of the families. An investigation of food and water sources will also be done.

3. Study of plasmid-mediated virulence and antibiotic resistance will be initiated.
   In 1981-82 the necessary laboratory methods will be established in the Bacteriology Research Division in order that actual research projects will be started in 1982-83.
   Plans for the study of colonizing factors will be drawn-up in 1981-82 so that methods may be established in 1982-83.

Supplies and equipment:

1. Most of the major equipment for the above studies including laboratory animals are in position and operational. Some essential equipment (eg. CO_2 incubator for tissue culture of CHO ce; tissue for identification of ETEC, etc.,) have been requested for and should arrive early.
2. Similarly reagents and chemicals have already arrived and are being used. However, some more supplies required for the large number of specimen to be processed according to work-plan for 1981-82 have been requested for.
3. Fquipment, reagent and laboratory supplies necessary for the above 3 work-targets for 1981-82 should receive first priority.

Japanese Experts:

1. Identification of the major pathogenic bacteria is the most important aspect of the project. Method of identification of Vibrio cholerae Salmonella, Shigella poses no problems. Identification of enteropathogic, enterotoxigenic (stable and labile) and enteroinvasive strains of E.coli have been set up. (except invasive).
   Method for the identification of others uncommon but known pathogens has also been set up (eg., Vibrio-parahemolyticus).
   However, the present results from pilot area have been able to identify known pathogens in 40-50% of diarrhoea stools. This is at variance with report from other reputable research centres (Dacca) using similar field and laboratory methods. Whether this difference is due to differences in sampling and methods or whether previously unrecognized organisms are causing diarrhoea in Burma needs to be clarified. It is highly unlikely that previously unrecognized organisms are responsible.

The Japanese Expert who comes should be very competent with these problems.
Also method for identification of ETEC (ST and LT) requires a time consuming method. Japanese expert should help set up an enzyme-linked-immuno assay system (ELISA) for this organism.
Methods for Plasmid research should be set up this year. The Head of Bacteriology Division (Dr. Daw Tin Aye) has been trained but will need help from Japanese Expert in setting up relevant methods.
Two months may be too short for Japanese Expert in helping with above and 4-6 months is preferable (if possible). It would be best if Japanese Expert has expertise in both identification of pathogens and plasmid research.

Burmese Trainee:
The technician Grade 1 been earmarked for training this year. However, instead of 1 x 12 months it would be preferable to send one Research Officer for 1 x 6 months. Because of the work-load both these persons may possibly be sent in later part of 1981.

Pathology Research Division

Work plan for 1980-1981

1. Immunofluorescence course given to members of relevent departments.
2. Some special staining techniques were established in the pathology research division.
3. Research:
   - 3.1 To establish methods for the study of "sludging" of red cells in the microcirculation during acute infections prevalent in Burma.
   - 3.2 Establishment of histochemical methods for the identification and localization of "kinins" in and around the cerebral microcirculation.
   - 3.3 Establishment of immunofluorescent and other techniques for the detection and location of immunocomplexes in the circulation and in tissues.
   - 3.4 Establishment of autoradiographic and electron-microscopic autoradiographic techniques.
   - 3.5 To plan and initiate a program of research in "Rheopheresis" and pathological alterations in blood flow in the microcirculation durimg acute infections prevalent in Burma.

Training:

1. Researcher level
2. Technician level

Pathology Research Division

Work plan for 1981 - 82

General objective :

The Pathology Research Division as one of the divisions participating in the DMR/JICA Research project, will study the role and importance of alteration in the microcirculation and clotting mechanism in the pathogenesis of important disease in Burma. (e.g., in dengue haemorrhagic fever) The general aim of the Pathology Research Division would therefore be to develop the capability to study defects in haemostasis, clotting and alterations in the microcirculation and apply them for investigation of pathogenetic mechanism in important diseases prevalent in Burma, in which such defects are considered to be of significance. (e.g., dengue haemorrhagic fever).

This would include development of expertise in ultra-structural study as an aid to the study of the above diseases.

Work Plan:

Histological and ultrastructural studies of the brain in an animal model and in human autopsy cases with defects in microcirculation and clotting mechanisms as a result of infection.

Training:

Local training in electron microscopic technique will be required for the two pathologists in the Pathology Research Division. This should be in the basic and routine techniques and should take about two to three months.

It is envisaged that pathological material from animal models and human autopsy cases for the research study will be available by the time the training start. This material would be used for training as well as to complete the research study mentioned above.

Expertise of Burmese counterparts:

The two researchers in Pathology Research Division have good experience ingeneral histopathology and clinical pathology. However, they will need training in special techniques such as electron microscopy.

There is very little previous experience in the study of the clotting defects.

Japanese experts:

A Japanese expert should help in the local training of the pathologist in electronmicroscopy techniques, and help with the ultrastructural studies that have been planned.

Burmese Trainee in Japan:

It is proposed that one pathologist be trained in Japan in all aspects of the study of clotting defects. On return, the trainee should be able to set up a clotting laboratory in the Division of Pathology and initiate studies on defects in clotting mechanisms related to infectious diseases prevalent in Burma such as dengue haemorrhagic fever. Period of training should be at least one year and it is proposed that the trainee be trained in a first class laboratory where routine as well as research on clotting disorders are conducted.

Supply and equipment:

1. Equipment and supply for general histopathology is available. Supplies for special techniques such as immunofluorescence are not yet available and will be requested for.
2. There are no supplies and equipment for studies on clotting defects.
3. Necessary supplies for the training in electronmicroscopy technique and works on ultrastructural studies will be required.

1. Effect of blood exchange on the histopathological lesions produced during heavy experimental malarial infection in animals (mice).

Scientific objectives:

1.1 The specific objective is to observe whether or not exchange of blood will remove or prevent the specific histopathological changes which accompany or precede death in heavily infected experimental models of malaria.

1.2 To observe whether mortality is reduced compared to controls, by such a procedure.

1.3 To infer whether mechanical changes play a significant and primary role in the sensation of death or the specific lesions, or whether play such a role.

Study design:

Phase 1 - Specific histopathological changes will be produced in experimental animals - Plasmodium berghei - mice system.

Phase 2 - Procedures for the exchange of blood in experimental animal (mice) or an organ will be set up.

Phase 3 - The effect of exchange on the specific histopathological lesion will be observed. Mortality rate in compared to controls will also be observed.

It is planned to finish phase 1 & 2 in 1981 and possibly phase 3.

Scientific justification:

(1) Certain histopathological changes have been observed in the brain and the cerebral microcirculation of human subjects who died of cerebral malaria.

(2) The significance of the observed histopathological changes with reference to their causal role produced in death or the specific clinical features of cerebral malaria is still debated.

(3) It has been postulated that exchange transfusion may prevent death in cerebral malaria by removing infected cells or the hypothetical toxins responsible for the histopathological changes and or death. A few uncontrolled clinical observation in mass suggest this possibility.

(4) There is as yet no firm basis for the postulate and it is our aim to (i) investigate whether there is experimental evidence to support this (ii) to use blood exchange as an investigation probe which might lead to better understanding of the pathogenetic mechanism in cerebral malaria similar to the way treatment with hydrocortisone has been used for study of pathogenetic mechanisms.

2. Histopathological changes in the cerebral, renal, hepatic and the pulmonary microcirculation in cerebral malaria.

Scientific objectives:

1. To observe whether the histopathological changes produced in microcirculation of the liver, kidneys and the lung are similar to the changes produced in the cerebral microcirculation.
2. To observe the changes in the chorpid plexus of the brain
3. To infer whether the changes in the endothelial cells and the basement membrane play any role in the pathogenesis of cerebral malaria.

Study Design:

Phase 1 - Experimental models of malaria would be produced in mice (P. berghei - mice system). Histopathological changes would be observed in the brain, kidney, liver, lungs. Particular attention would be paid to the change in the vascular system. Appropriate special stains would be used.

Phase 2 - Specimens of brain, kidneys, liver and lungs obtained at post-mortem would also be examined. It is planned to finish phase 2 if an adequate number of post mortem specimens are received.

Scientific justification:

It has been stated that in malaria, a chain of reaction takes place in the host. The initial reaction is thought to be due to a change in the permeability of the blood vessels.

(1) Only a few studies have been made on the histopathological changes of various organs. (2) None have studied the changes in the choroid plexus, the ependymal lining of the ventricles, nor the changes in the microcirculation. (3)Comparisons were thus not made on the changes produced in the blood vessels.

We aim to fill the gaps in knowledge and to also observe whether cerebral malaria is due to any specific changes found in the cerebral microcirculation only.

3. Coagulopathies in cerebral malaria in collaboration with Lt/col: Dr. U Mya Oo, (DSGH) (principle investigator).

3.1 Objectives:

3.1.1 To observe whether coagulation disturbances namely intravascular coagulation is present in cerebral malaria only compared to the non-cerebral controls.

3.1.2 To see whether IV coagulation play a role in the pathogenesis of C.malaria.

3.2 Study Design:

- Blood samples will be obtained from the DSGH.
- Special tests will be done - to detect the presence or absence of I.S.C.

Planned to finish in 1981.

3.3 Justification:

The role of I.S.C in the pathogenesis of cerebral malaria is still uncertain. Some regard it as a terminal event while others claim as the "initial" reaction. A lot of work has been done and still the presence or absence of Intra.Venous coagulation in cerebral malaria is controversial.

Using a large series of cases, we aim to see the presence or absence of Intra venous coagulation in our subjects, and to see whether death in cerebral malaria is due to the I.V.C.

Immunology Research Division

Work plan for 1981 - 82

In accordance with the minutes of the 1st Coordination Committee meeting, the Immunology Research Division of DMR is planning to set up and establish hybridoma technology with an aim to use this method in future particularly in research and control of infectious diseases. Establishment of the technology in Immunology Division is desirable and it is also feasible with the present staff and equipment provided necessary materials are available.

Research objective for 1981-82

In this initial year the main target is setting up of cell hybridization method in the Division. Known myeloma cell line to be obtained from abroad will be maintained in long-term culture. Hybridization with antibody producing cells from mouse will be done with the myeloma cells and the method of characterization of antibodies produced will be established initially with prototype immune cells producing well-known antibodies (e.g., anti-SRBC).

Requirement of a Japanese Expert:

Cell hybridization being a relatively new technology the staff of the Immunology Research Division has no special training nor previous practical experience. They should be given practical training in the working details of the cell hybridization technology. A Japanese Expert of high standing who is well-trained and presently working with long-term cell culture and cell hybridization procedure will be required to stay in DMR for at least 3 months to give reasonably adequate practical training to the staff of the Immunology Research Division.

The present Senior Research Officer (Dr Khin Ohn Lwin) of the Division had training and experience in general immunological methods and cell-mediated immunity and hence would be familiar with various techniques (apart from cell hybridization) ... required in the various stages of hybridoma technology e.g.,

antibody characterization. A new Head of Immunology Division will also be appointed in about 3 months time. The SRO and new Head of Division can act as counterparts to the Japanese Expert in development and establishing of hybridoma technology in DMR.

For the new head of division to be trained and get involved in the establishedment of hybridoma technology, the Japanese Expert should start the work by about November 1981. (The head of the Division will be attending hybridoma course in Singapore in the middle of October 1981 for one week).

Materials required

The major equipment required are already available in Immunology Research Division, except for the gas sterilizer which will be required for sterilization of plastic materials used in cell culture. The gas sterilizer as well as other materials and reagents required for setting up of hybridoma method are already indented under DMR-JICA Colloborative Plan.

Laboratory Animal Service

Work plan for 1981 - 82

Work objectives:

1. To maintain quality breeding of laboratory animals and produce enough for the needs of DMR.
2. To enable experimental animal section to be used by researchers.
3. Genetics and Disease Control capability will have to be developed slowly as and when new staff are appointed later this year.

Supplies and Equipment:

Necessary supplies and equipment to be procured from 1981-82 have been requested for.
Food pelleter is essential.

Japanese Experts:

It is proposed that one Animal Expert (general) come to Burma in 1981-82. It is too early for Experts in Disease and Genetics because new staff to be trained in these subjects will be appointed only later this year.

Annex - 3

Japanese experts

(1980 - 1981)

	Name designation	Subject	Date	Period
1.	Professor Y. Hamashima Professor, Kyoto University.	Pathology	30/10/80 to 29/12/80	2 months.
2.	Dr. H. Hayashi Associate Professor, Okayama University.	Bacteriology	1/11/80 to 29/4 /81	6 months.
3.	Dr. M. Nakagawa Head researcher of animal laboratory, National Institute of Health.	Laboratory animal services	21/10/80 to 21/12/80	2 months.
4.	Professor A. Ohyama Professor, Osaka University.	Virology	16/12/80 to 13/2 /81	2 months.
5.	Professor Y. Kanemasa Professor, Okayama University.	Bacteriology	19/6 /81 to 8 / 8/81	2 months.
		1981 - 82		
1.	Professor Y. Hamashima Professor, Kyoto University.	Pathology	15/12/81 to 19/1 /82	1 month.
2.	Professor A. Ohyama Professor, Osaka University.	Virology	17/12/81 to 24/1 /82	$1\frac{1}{4}$ month
3.	Professor K. Nakane Professor, Tokei University.	Pathology Electron Microscopy	16/1 /82 to 29/1 /82 16/2 /82 to 15/3 /82	$1\frac{1}{2}$ months.

1981 - 82

Name designation	Subject	Date	Period
4. Dr.T.Asano Researcher, National Institute of Health.	Laboratory animal services	19/1 /82 to 17/3/82	2 months.
5. Dr. H. Hayashi Associate Professor, Okayama University.	Bacteriology	19/1 /82 to 10/3 /82	$1\frac{3}{4}$ months.
6. Dr. T. Ito Associate Professor, Osaka University.	Virology	24/1 /82 to 23/4/82	3 months.

Annex - 4

Burmese trainees in Japan

Name	Educational Qualification	Position in DMR.	Subject of training	Period
		(1980 - 81)		
1. Mg Aung Myint	B.Sc(Chemistry)	Technician Grade (1). Virology Research Division	Virology technology	1 year.
2. Mg Myint Soe	B.E.	Research Officer, Instrumentation Division.	Instrument maintenance and repair.	1 year.
3. Myat Myat Thu	M.Sc (Zoo).	Technician Grade (1). Entomology Research Division.	Entomology technology	1 year.
		(1981 - 82)		
1. Dr. Than Than	M.B.B.S, Ph.D	Head, Pathology Research Division.	Pathology Research	1 year.
2. Dr. Kyi Kyi Khin	M.B.B.S,D.Bact.	Senior Research Officer, Virology Research Division.	Virology Research	1 year.
3. Khin Sann Aung*	B.Sc (Zoo).	Technician Grade (1). Bacteriology Research Division.	Bacteriology technology	1 year.

*Tentative

Burmese trained in Japan by JICA before project implementation but in anticipation of Project.

(1977 - 1978)

Name	Educational Qualification	Position in D.M.R.	Subject of training	Period
1. U Soe Myint	M.Sc.	Senior Research Officer, Instrumentation Division.	Instrument maintenance and repair.	1 yr.
2. Khin Mg Zaw	B.Sc.(Zoo).	Technician Grade (1). Animal laboratory service.	Laboratory animal technology.	1 yr.

Annex - 5

LIST OF ACADEMIC ACTIVITIES (1980-82).

Participation in International workshopsand seminars

(relevant to the Project).

1. Dr. U Tun Pe, (Head, Immunology Research Division), and U Thet Win (Research Officer, Virology Research Division) attended the regional workshop on "Symposium on the properties of the monoclonal antibodies (produced by hybridoma technology) and their application to the study of diseases" held at the National University of Singapore from 19 - 23, October 1981.

2. Dr. Daw Khin Ohn Lwin, (Senior Research Officer, Immunology Research Division) attended the training course on "Derivation of hybridoma-producing monoclonal antibodies" at Chiba University School of Medicine in Chiba, Japan from 26 October to 4 November, 1981. It was sponsored by the Asian Molecular Biology Organization.

3. Dr. Mi Mi Khin, (Deputy Director, Research) attended the International Seminar on Viral diseases in South East Asia and the Western Pacific, sponsored by the Australian Academy of Science in Canberra, Australia, from 8 - 12, February 1982.

4. Virology and Entomology Research Divisions of the Department of Medical Research will host the WHO Workshop on "Intercountry Workshop on Mosquito Inoculation and Tissue Culture Techniques for Arboviruses Isolation" in March - April, 1982.

5. The Second Asian Conference on Diarrhoeal Disease to be held in Calcutta, India, in February 22 - 25 1982, will be attended by Dr. Daw Tin Aye (Head, Bacteriology Research Division) and Dr. Khin Maung U, (Head, Clinical Research Division).

6. Dr. Mi Mi Khin (Deputy Director, Research) attended the Scientific Group Meeting on "Rapid diagnostic tests for viral infections" held at the WHO Headquarters, Geneva, Switzerland, from 29 September to 4 October, 1980.

7. Dr. Khin Maung Tin (Deputy Director, Research) attended the Regional Advisory Committee on Medical Research Meeting on Dengue Haemorrhagic Fever held in New Delhi, India, from 30 March to 31 March 1981.

11. Workshops, scientific meetings and talks at the Department of Medical Research

(Relevant to the Project)

1. A Workshop on ELISA methodology, 12-13 January 1981.

2. Dr. H. Hayashi (21-2-81)	: Structure and function of erythrocyte membrane.
3. Dr. Y.Kanemasa (25-7-81)	: Phospholipid composition of Staphyloccus aereus.
4. Dr. David A.Sack (30-9-81)	: Recent advances in Research on etiology and therapy of acute Diarrhoea.
5. U Thet Win (3-10-81)	: Detection of Dengue virus by immunofluorescence after Intra-cerebral inoculation of Toxorhynchites splendens mosquitoes from mononuclear cells of Dengue haemorrhagic fever patients.
6. U Khin Mg Zaw (17-10-81)	: Breeding of Nude mouse at DMR animal house.
7. Dr. Y.H. Bang(24-10-81)	: Applied research for National Vector Borne Disease control programmes.
8. Dr. Daw Tin Aye (5-12-81)	: Experience in International Centre for Diarrhoeal Diseases Research.
9. Dr. Tun Pe (20-1-82) Dr. Daw Khin Ohn Lwin U Thet Win	: Hybridoma
10. Professor K. Nakane (23-1-82)	: Peroxidase labelled antibody method.
11. Dr. H. Hayashi(21-3-81)	: Current review on the basis of immune reaction.
12. Daw Mi Mi Khin(27-12-80)	: Detection of Dengue antigen in LLCMK/2 Cell lines.

111. Scientific meetings and talks at the Department of Medical Research

(on other subjects)

1. Dr. Myo Thein (7-3-81) : Stamina
2. Dr. Aung Khin (4-4-81) : The strategy of life
3. Ma Phyu Phyu (18-4-81) : Protective action of G-6-PD deficiency against malaria.
4. Daw Thawka Kyin (16-5-81) : Induction of immunity in mice by Ascaris suum eggs.
5. Dr. Ne Win (6-6-81) and Dr. Khant : Recent trend in drug abuse
6. U Tun Khin (13-6-81) : Quality control in medical laboratory technology.
7. Dr. B. Murphy (20-6-81) : Laboratory diagnosis of viral hepatitis.
8. Dr. Maung Maung Oo(4-7-81) : Basic Principles of calculation in digital computers.
9. Prof: R.L. Smith (17-7-81) : Pharmacokinetics and drug metabolism.
10. Dr. Y. Kanemasa (25-7-81) : Phospholipid composition of Staphylococcus aureus.
11. Dr. R.D. Piyasena(15-8-81) : Recent testicular function control-recent-research development.
12. Dr. Willougby Tun Lin(5-9-81): People's Health programme in Burma.
13. Daw Htay Htay Aye(7-11-81) : Practical use of calculators in Biomedical Research.
14. Dr.Alton I.Sutnik(30-11-81): Hepatitis B and liver cancer.
15. Dr. Y Sawai (8-12-81) : Recent advances in pathogenesis and medical treatment of snake-bite.
16. Dr. Thein Hlaing(19-12-81) : Conceptual epidemiological model of ascariasis.

17. Daw Than Saw(24-1-81) : Application of different preparations of Ascaris suum antigens to the ELISA test.

18. U Mg Mg Thwin (3-1-81) : The use of constant infusion technique in studying the effect of irradiation on ^{3}H-uptake in mice.

19. U Phone Myint(27-12-80) : Decreased Serine Hydromymethylase Activity as a cause of Defective DNA Synthesis in Iron Deficiency.

20. U Thein Than (20-12-80) : Development of an in-vitro system for testing of food iron absorption.

21. Prof: Hamashima(20-12-80) : Kawasaki Disease

22. U Myint Lwin (13-12-80) : Future prospects of Malaria vaccine.

23. Prof: R. Ba Pe (4-12-80) : Clinical trial of indigenous bronchodilator drugs.

24. Dr. Soe Soe Aye (4-12-80)
Dr. Mu Mu Khin : Clinical trial of chlorpromazine as anti-secretory agent.

25. Daw Aye Aye Myint(29-11-80) : Serial measurements of snake venom concentrations in Experimental Animals by ELISA after envenomation: its implications and future use.

Postgraduate students conducting Research in topics relevant to DMR/JICA Project or in Divisions strengthened by this Project

(1980 - 1992)

1. Aye Aye Myint : (1980-81) M.Sc (Zoology).
Rangoon University.

Title of Thesis : Establishment, Standardization and Application of ELISA in Russell's Viper evencmation.
(Immunology Research Division, DMR).

2. Dr. Phyu Phyu Win : (1980-to date) M.Sc (Microbiology).
Institute of Medicine, Mandalay.

Title of Thesis : A Study of Cell-mediated Immunity in Leprosy Patients.
(Immunology Research Division, DMR).

3. Dr. Soe Thein : (1981-to date) M.Med, Sc. (Microbiology).
Institute of Medicine, Mandalay.

Title of Thesis : Viability of JE virus in glyceronized mosquitoes and tissues.
(National Health Laboratory & Virology Research Division, DMR).

4. Khin Mar Aye : (1981-to date) M.Sc (Zoology).
Rangoon University.

Title of Thesis : Virulence and temperature sensitivity of Dengue viruses.
(Virology Research Division, DMR).

5. Dr. Mya Mya Ohn : (1981-to date) M.Med, Sc. (Paediatrics).
Institute of Medicine (1), Rangoon.

Title of Thesis : Neonatal diarrhoea - major etiological agents & mortality patterns.
(Clinical & Bacteriology Research Division, DMR).

Annex - 6

Revelant papers presented at scientific meetings by Scientists working in the DMR/JICA Project

1. Ohyama A., Thet Win & Tanimura E. (1981). Isolation and identification of Dengue virus (Type 11) by Toxorhynchites mosquitoes. The 23rd Annual Meeting of Japanese Society of Tropical Medicine, 1981 Tokushima, Japan.

2. Ohyama A., Thet Win & Tanimura E., (1981). Development of Dengue virus in Toxorhynchites mosquitoes after intracerebral inoculation technique. The 29th Meeting of Japanese Virologists, 1981 Tokyo, Japan.

3. Ito T., Tanimura E., Tamamoto N., Nakamichi A., Aung Myint & Ohyama A., (1981). Quantitative detection of Dengue and Rubella Viruses using cultured cells. The 29th Meeting of Japanese Virologist, 1981 Tokyo, Japan.

4. Khin Maung U, (1980). Laboratory and Field studies in Diarrhoea of Adults and Children in Tropical Climates. Presented at Clinical Gastro enterology Meeting on 12-12-80 at the MRC Clinical Research Centre, Harrow, Middle sex, England.

5. Khin Maung U, (1981). Intestinal Transport Studies of Calcium and Phosphate a model for ionic transport study in diarrhoea. Presented at Clinical Cell Biology Seminar on 3-4-81 at the MRC Clinical Research Centre, Harrow, Middle-sex, England.

6. Khin Maung U, (1981). Operational Research Methodology for domiciliary oral rehydration of acute diarrhoea among village children in Burma and its effect on their growth and nutrition, Presented at Harvard University School of Public Health on 7-5-81 Boston, Massachusetts, USA.

7. Khin Maung U, (1981). Effect of oral rehydration on growth and nutrition in rural Burma. Presented at International Food and Nutrition Programme Seminar, on 7-5-81 United Nations University, Massachusetts Institute of Technology, Boston, Massachusetts, U.S.A.

8. Khin Maung U, (1981). Operation Research Method to study feasibility, acceptability and effectiveness of oral rehydration for acute diarrhoea among village children by their mothers at home, and its effect on their growth and nutrition. Presented at Inter-University School of Medicine, Baltimore, Maryland, U.S.A

9. Mi Mi Khin, (1980). Developement of rapid laboratory diagnostic test for Arboviral infections. Participation as Member Scientific Group Meeting on developement of rapid laboratory techniques for viral infections. 29th Sept: - 4 Oct: 1980 at WHO, HQ, Geneva, Switzerland.

10. Mi Mi Khin, (1982). Viral infections in Burma. To present at the International Seminar on Viral Diseases in South East Asia and the Western Pacific. 8 Feb: - 12 Feb: 1982 at Canberra, Australia.

Annex - 6

Relevant papers published by Scientists working in the DMR/JICA Project

1. Thet Win & Ohyama A., (1981). Detection of Dengue - 2 prototype virus in Toxorhynchites mosquitoes using intracerebral inoculation techniques. Dengue Newsletter, Southeast Asian and Western Pacific Regions (W.H.O.) 7: 51 - 52.

2. Thet Win, (1982). Detection of dengue virus by immunofluorescence after intracerebral inoculation of mosquitoes. The Lancet, January 2, 1982. 53 - 54.

3. Mi Mi Khin, (1982). Transovarial transmission of dengue virus by Aedes aegypti in nature. (sent for publication American J. Trop. Med. & Hyg).

4. Sebastian A., Myat Myat Thu, May May Kyaw & Myint Myint Sein, (1980). The use of dragon fly nymphs in the control of Aedes aegypti. Southeast Asian J. of Trop. Med & Pub. Health., 11: 104 - 107.

Annex - 6

Papers presented on other topics by Scientists working on DTT/JICA Project

1. Aung Khin M., (1980). The problem of Snake bites in Burma. Paper was presented by Dr. Aung Khin, Head of Immunology Research Division, D.M.R, at International Seminar on Snakebites. 25 - 28 August 1980. Okinawa Island, Japan.

Annex - 6

Papers published on other topics by scientists working on DMR/JICA Project

1. Noguchi U, Mar Mar Nyein, Kohsaka K, Yoneda K, and Mori T, (1980). The growth and drug sensitivity of M. Lepraemurium by tissue culture applying monolayer and agar suspension technique. International J. of Leprosy. Vol. 48, 277 - 284.

2. M. Aung Khin, Khin Ohn Lwin and Thant Zin, (1980). Immunogenicity of the toxoid of Russell's Viper Venom. The Snake, Vol. 12, P.P. 45 - 53.

3. Aung Khin, M., (1980). The problem of Snake bites in Burma. The Snake 12. (1.2) 109.

4. M. Aung Khin, Khin Ohn Lwin and Thant Zin. (1980). Active Immunization with Refined Toxoid of Russell's Viper venom. (1) Study of Immunogenicity in monkeys and other animals. Amer J. Trop. Med. and Hyg. (sent for publication, not yet accepted).

5. M. Aung Khin, Khin Ohn Lwin and Thant Zin, (1980). Active Immunization with Refined Toxoid of Russell's Viper venom. (11). Study of immune response in human volunteers. Amer J. Trop. Med. and Hyg. (sent for publication, not yet accepted).

6. Aye Kyaw, (1982). Partial purification and some properties of Caseinolytic activity in Russell's Viper venom. The Snake Vol. 14 (1), 1982.

7. Thane Toe and Thane Than, 1982. Serum Ferritin Studies in Burmese Pregrant women. American Journal of Clinical Nutrition, Vol. ( ), January issue.

8. Thane Toe, 1981. Iron Supplementation in Pregrant women. Report to WHO.