ビルマ国消化器感染症
研究プロジェクト
長期調査員報告書

昭和60年９月

国際協力事業団

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序　　文

ビルマ国の医学研究に対し，わが国は，昭和４２年より５年間，医学研究所においてウィルス部門の協力を，また，昭和５５年より4年間，わが国の無償金協力により完成した生物医学研究センターにおいて，ウィルス学・細菌学・免疫学部門のプロジェクト方式技術協力を実施し，それぞれ多大な成果を収めた。

ビルマ国政府は，この研究成果をさらに発展・向上させるため，新たに肝臓及び消化器管の細菌性，ウィルス性及び寄生虫性疾患の研究に対する協力を要請，これを受けて昭和６０年２月事前調査団が派遣された。

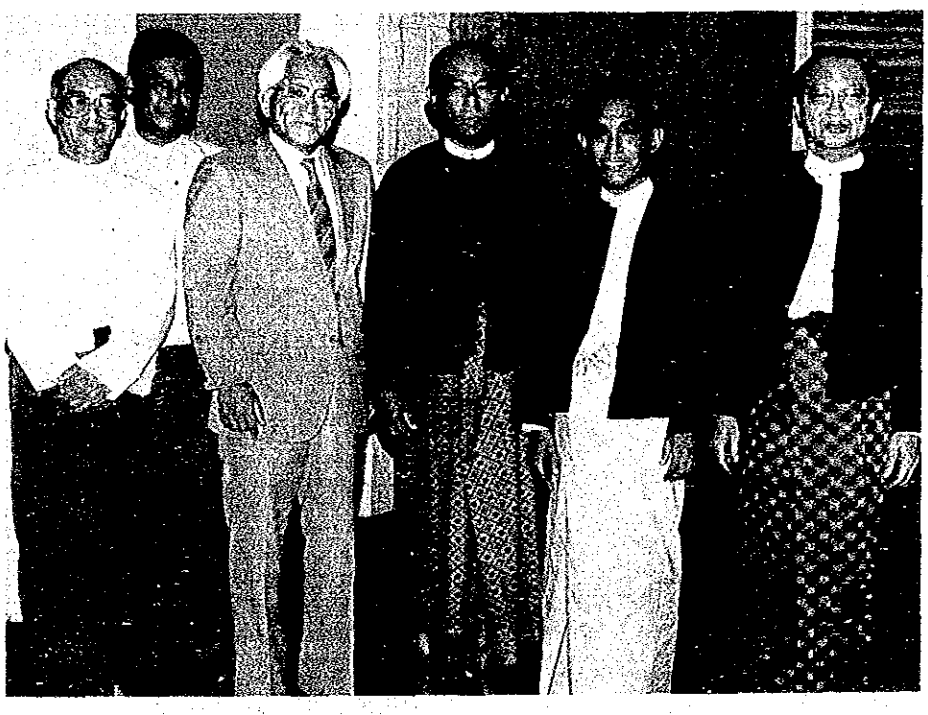
事前調査の結果，本プロジェクト実施の必要性が確認されたが，協力計画作成のため，協力内容をさらに明確に把握する必要があることから，当事業団は，昭和６０年８月１１日より同２５日まで，長期調査員２名を派遣した。本報告書は，その調査結果をまとめたものであり，ここに調査員各位並びに同調査員派遣にご協力いただいた関係機関の方々に対し，深甚なる謝意を表するものである。

また，同国に対する医療協力の実施について，今後とも関係者各位のご理解とご協力をお願いする次第である。

昭和６０年９月

国際協力事業団
理事　末永昌介

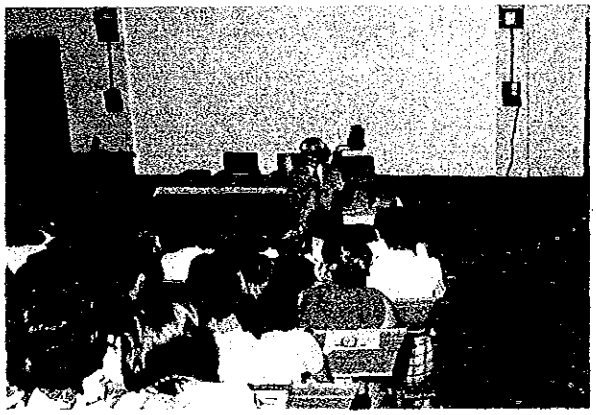
ビルマ大統領 U San Yu 氏（左）と懇談する濱島教授。右は保健大臣 U Tun Wai 氏。大統領官邸において。昭和６０年８月１３日。



ビルマ国保健行政首脳陣。
中央　U Tun Wai 保健大臣。
向かって左より右へ。
Dr. Pe Thein 医学教育局長。
Dr. L. Aung Din スポーツ局長。
濱島教授。
保健大臣。
Dr. Aung Than Batu 医学研究局長。
Dr. Tin Oo 保健局長。

新プロジェクトに基づいた研究実施方針をビルマ側研究者と打合せ。
於：ビルマ国立生物医学研究センター集会室。
昭和６０年８月１３日。

Non A－Non B 肝炎研究を協議する。
左より，志方教授，Aung Than Batu 局長，
濱島教授。



ビルマ国立第一医学校で学生，医師に講演する濱島教授。

志方教授の学術講演。Mandalay 医学校にて。
昭和６０年８月１９日。Dr. Hla Myint の質問を受ける志方教授。

目　　次

Ⅰ　長期調査員派遣の経緯

ビルマ国政府は，同国における医学研究レベルの向上のため，わが国との従前の技術協力の成果を継続発展させることを目的として，昭和５９年，新プロジェクトとして，PROJECT OF RESEARCH ON TREATMENT OF INFECTIOUS DISEASES OF THE ALIMENTARY SYSTEM の要請を提出した。これに対し，国際協力事業団は，昭和６０年２月，濱島義博京都大学医学部教授を団長とする事前調査団を派遣，要請内容の検討を行った結果，本件技術協力の実施が妥当である，との結論に達した。

本プロジェクトのテーマの一つに，肝炎の研究が挙げられているが，このテーマは，今回の技術協力において，極めて重要な位置を占めることから，ビルマにおける肝炎の実態調査等を通じ，同分野における協力の内容・範囲を確定するため，実施協議に先立ち，長期調査員が派遣されることとなった。

Ⅱ　長期調査員の構成と調査日程

Ⅱ－１　長期調査員の構成

濱　島　義　博
京都大学医学部教授（病理学）

志　方　俊　夫
日本大学医学部教授（病理学）

Ⅱ－２　調査日程

期間　昭和６０年８月１１日（日）～
同８月２５日（日）　　１５日間

月　日	曜日	内　　容
８月１１日	日	１６：２０　TG７４１便　成田発
		２０：３０　Bangkok着
１２日	月	１２：１０　Royal Orchid Hotel発
		１４：５０　TG３０５便　Bangkok発
		１５：２０　Rangoon着
		空港にオンタンバツー局長，研究所スタッフ多数，篠浦所長，羽根田医務官夫妻らの出迎えを受ける。
		１７：００　Inya Lake Hotel着

月　日	曜日	内　　容
8月13日	火	9：15　日本大使館に塚本大使表敬訪問。今回訪緬の目的を説明。
		10：00　DMR。局長と第一回打合せ。
		11：00　志方教授DMR各部局視察。
		13：30　DMRカンファレンス室にてDMR研究実情の報告会ならびに各部より新プロジェクトにおける研究計画発表。次いでR／D草案の検討。
8月14日	水	9：00　新プロジェクトに関する研究計画の打合せ。
		14：00　U San Yu 大統領の招きにより，濱島教授，大統領官邸訪問。U Tun Wai 保健大臣と3人で約1時間半懇談。
15日	木	9：00　保健局訪問。U Tin Oo 局長と1時間半面談。(消化器病プロジェクトについても新研修員（4名）のA2・3Form，機材A4Formの修正など依頼。)
		11：00　医学教育局 Pe Thein 局長を訪問，日本政府受入れのビルマ医学教育視察団3名の要請リストA 2・3 Form を急ぎ出すよう要請。
16日	金	9：30　第一医学校訪問。キン　チチ　微生物学教授からビルマの肝炎ウィルスの実情について聞く。
		14：00　ラングーン新病院の月例症例検討会に出席。
8月17日	土	休日
18日	日	6：50　ラングーン空港発
		9：20　パガン着。直ちに4カ処の部落訪問。原地人生活実態と肝炎調査を行う。Thiripyitsaya Hotel 泊。
19日	月	8：45　パガン発。
		9：15　マンダレー着。直ちにマンダレー医学校へ。
		10：00～12：00　講演。
20日	火	7：00　サガイン　ヒル附近の住民生活環境の視察。
		13：00　マンダレー医学校病理学教室スタッフと会議。
		15：45　マンダレー発。
		17：30　ラングーン帰着。
21日	水	10：00　U Tun Wai 保健大臣と約1時間半面談。
22日	木	8：40　オン　タン　バッー局長室にて“Memorandum”の最終打合せ。
23日	金	8：30　“Memorandum”の最終タイプ。
		11：00　日本大使館に塚本大使を訪問。成果を報告する。

月　日	曜日	内　　容
		１９：００　保健大臣　U Tun Wai 氏および４局長と会議。
２４日	土	１６：２５　ラングーン発
		１８：０５　バンコック着
		Royal Hotel 泊
２５日	日	１２：３０　定刻より１時間半遅れてＴＧ７４０便でバンコック離陸。
		２０：００　成田着　帰国。

Ⅱ－３　関係者氏名一覧

１）日本大使館

塚　本　政　雄	大使
篠　浦　　烈	ＪＩＣＡ事務所所長
高　嶋　俊　政	ＪＩＣＡ事務所所員
羽根田　　敏	医務官
羽根田　やえ子	消化器病プロジェクト派遣専門家

２）国家計画財務省

U Antt Kyaw	Director General, FERD
U Hla Pe Than	Asst. Director, FERD
U Kyaw Tint	Additional Director

３）保健省

U Tun Wai	保健大臣
Dr. Aung Than Batu	Director General, Dpt.of Medical Research
Dr. Tin Oo	Director General, Dpt.of Health
Dr. Pe Thein	Director General, Dpt.of Medical Education
Dr. Aung Tin	Director General, Dpt.of Sports
U Set Maung	Director General,FERD

DMR

Dr. Kywe Thein	Deputy Director
Dr. Mi Mi Khin	Deputy Director

BACTERIOLOGY RESEARCH DIVISION

— Head — Dr. Tin Aye

— Daw Mar Mar Nyein

— Dr. Phyu Phyu Win

— Dr. Khin Nwe Oo

BIOCHEMISTRY RESEARCH DIVISION

— Head — U Hla Pe

— U Tin Win

CLINICAL RESEARCH DIVISION

— Head — Dr. Khin Maung U

— Dr. Nyunt Nyunt Wai

— U Aye Kyaw

— Dr. Myo Khin

— U Tin Oo

EXPERIMENTAL MEDICINE DIVISION

— Head — Dr. Hla Myint

— Dr. Khin Maung Win

— Dr. Daw Tin Nu Swe

— U Tun Khin

IMMUNOLOGY RESEARCH DIVISION

— Head — Dr. Tun Pe

NUCLEAR MEDICINE DIVISION

— Head — Dr. Thein Than

— U Mg Mg Thwin

PARASITOLOGY RESEARCH DIVISION

— Head — Dr. Myint Lwin

— U Myint Oo

— Daw Than Saw

— Dr. Ye Htut

PATHOLOGY RESEARCH DIVISION

— Head — Dr. Daw Than Than

— Dr. Mg Mg Oo

— U Thet Win (1)

— Dr. Khin Ei Han

— Dr. Daw Soe Soe

VIROLOGY RESEARCH DIVISION

— Head — Dr. Soe Thein

— Dr. Kyaw Moe

— U Thet Win (2)

— Dr. May La Lin

EPIDEMIOLOGY DIVISION

— Dr. Daw Myint Soe

ANIMAL HOUSE

— U Khin Maung Zaw

INSTRUMENTATION

— Head — U Soe Myint

— U Myint Soe

— U Htay Aung

Health

Dr. Maung Kyaw (Director)

Capt. Kyi Soe (P.A. to Deputy Minister)

U Tin Nyunt (P.A. to Minister)

Dr. Aung Myint (Deputy Director)

Medical Education

Prof. Hla Myint (Rector, Institute of Med. 1)

Dr. May May Yi (Director)

Dr. Thane Toe (Deputy Director)

Dr. Thein Maung Myint (Deputy Director)

Hospital

Dr. Khin Maung Gyi (Medical Superintendent)

Dr. Win Maung (Prof. of Anaesthesia)

Dr. Maung Maung Lay (Prof. of Surgery)

Dr. Ba Pe (Prof. of Medicine)

Dr. Tun Yi (Prof. of Radiology)

Dr. U Maung Ko (Head of Pathology)

Dental College

Dr. Khin May Lay (Rector)

Mandalay

Dr. Tun Tin (Rector)

Dr. Khin Maung Than (Superintendent)

Dr. Khin Maung Win (Prof. of Medicine)

Dr. Ohn Swe Mo (Blood bank)

Dr. Kyaw Sein (Administrator)

Dr. Hla Oo (Prof. of Pathology)

Dr. Daw Saw Mya Yee (Pathology)

Dr. Daw Khin Aye Kyi (Pathology)

Dr. Daw Hla Min (Consultant Pathologist)

Dr. Khin Maung Yin (Consultant Pathologist)

Ⅲ　調査概要と総括

Ⅲ－1　調査目的

本調査は新しく要請の出た，ビルマ国保健省医学研究局生物医学研究センターを中心とするプロジェクト"Research on Treatment of Infectious Diseases of the Alimentary System"に対する長期調査である。本プロジェクトの内容は，肝臓および消化管の細菌性，ウイルス性ならびに寄生虫性感染症の研究を中心とするというものであり，従来からのすぐれた研究成果をさらに飛躍的に発展させる上で重要なプロジェクトとして，日本側もこれを前向きに検討することとなつた。とくに，ビルマ国の主要疾患の一つである乳幼児下痢症は極めて猛毒をもつた毒素原性病原性大腸菌こそがその主犯であるという極めて重要なことが永年にわたる技術協力の結果で判明したことや，デング一熱ウィルスの分子生物学レベルでの毒性抗原因子の発見など，中には国際レベルにまで達する程の研究成果の上がつて来ている事実からして，この漸く緒についたばかりのビルマの医学研究レベルを，今こそさらに一大進歩を遂げさせるべく，技術協力を継続することの如何に大切であるかということが，前回の事前調査団によつて力説されたのである。

しかるにこの新しく要請の出たプロジェクトの項目の中には，肝炎，特にウィルス感染性の肝炎の技協が含まれていることが注目されるに到つた。何故ならば肝炎研究のプロジェクトというものは，現在，わが国においても大変重要なプロジェクトの一つであり，欧米文明諸国でも目下，肝炎の研究には異常なまでの努力を惜しまないのが現状である。ましてや開発途上国においては，すべての国に共通してこのウィルス性肝炎という疾患は重要な研究対象であり，ワクチンなどの早急に要求される，人類にとつて重要プロジェクトの一つなのである。

一口にウィルス性肝炎のプロジェクトと云つても，その方針の次第によつたり考え方によつては，極めて巨大なプロジェクトたり得るものであることから，このような大きな研究項目の加わつているこのプロジェクトは，最大限の慎重さを以て調査することが必要となつた。

ビルマにおいてもし，この新プロジェクトのもとでウィルス肝炎の技協を実施することとなれば，ビルマにおいて実地に，どれだけの範囲の研究が望まれ，かつ可能なのか，限られた期間と予算で果してこのような研究の技協をこの際するのが妥当なりや否や，実際に行うものとしてその具体的な研究計画がどのように立てられるか，またそうした場合には莫大な費用が要求されるのではなかろうか，などという懸念から，今回は，この肝炎の問題に絞つて事前調査の補足検討を行う必要に迫られたのである。その目的のために，ウィルス性肝炎病理の世界第一人者，日本大学志方俊夫教授に御足労願つて，つぶさに実地を検討しそのウィルス性肝炎研究の可能性について調査して頂くのが今回の主たる目的である。

Ⅲ－2　調査実施の概要

本調査は昭和６０年８月１２日より同８月２４日の間，討議と実地の両調査を行い，討議は主

としてラングーン医学研究局生物医学研究センター会議室で，実地調査は肝炎多発地区であるラングーン郊外のアロン地区ならびにタケダ地区，さらにUpper Burmaのマンダレー　サガイン地区とパガン郊外で住民の生活環境状態を主として行った。

8月13日（火）

6：50　濱島，志方両団員の早朝打合せ。

9：15　日本大使館，塚本大使表敬。

10：00　ビルマ国保健省医学研究局生物医学研究センターカンファレンスルームにおいて，新プロジェクトに関する実施計画の討議を開始。13：00より各部主任，関係Dr．全員集合して，先ず最近の彼らの得ている研究成果の報告およびこのプロジェクトに対する各自の研究計画の発表などから始めて貰い，この新しいプロジェクトの計画を立案する基本を得ることに努めた。

この会議にはJICAラングーン事務所の篠浦所長，高嶋所員も参加したが，終始，医学研究の専門的な討論の連続であった。

①Dr．Hla Myint（実験医学科主任）：Non A・Non B ウィルスとNon A・Non B肝炎の研究目的。

②Dr．Khin Maung Win（実験医学科）：妊婦肝炎患者の高死亡率。

③Dr．Soe Soe（病理科）：肝炎の病理

④Dr．Than Than（病理科主任）：血管内凝固の病因。

⑤Dr．Mi Mi Khin（ウィルス科，副所長）：ロータウィルスのAvidin－Biotin Immunoassay。

⑥Dr．Myint Lwin（寄生虫部主任）：赤痢アメーバとその肝，消化管感染。

⑦Dr．Tin Aye（細菌学部主任）：急性下痢症の細菌学的検査。

以上の研究発表のあったのち，各々についての質疑応答がなされた。

この新しいプロジェクトの技術協力の骨子は，肝炎のみに終始するものではなく，肝炎の研究は新プロジェクト研究全体の一部であること，主体はあくまでも，過去17年間築き上げて来たすぐれた成果の微生物学，ウィルス学，免疫病理学などの研究継続であって，これに新しく肝炎という極めて重大なテーマが加わったものであるとの理解が両チーム間で了承された。

会議出席者リスト

Burmese side

Dr．Aung Than Batu，Director General

Dr．Kywe Thein，Deputy Director

Dr．Mi Mi Khin，Deputy Director

Dr．Khin Maung Win，SRO．，Exp．，Med．

Dr．Hla Myint，Head，Exp．，Med．

Dr．Than Than，Head，Pathology

Dr. Soe Soe, SRD, Pathology

Dr. Mg Mg Oo, SRD, Pathology

Dr. Soe Thein, Head, Virology

Dr. Kyaw Moe, SRD, Virology

Dr. Tin Aye, Head, Bacteriology

Dr。Phyu Phyu Win, RO, Bacteriology

Dr. Mar Mar Nyein, SRD, Bacteriology

Dr. Tun Pe, Head, Immunology

U Hla Pe, Haed, Biochemistry

Dr. Myint Lwin, Head, Parasitology

Daw Than Saw, SRD, Parasitology

U Tun Khin, SRD, EM

Dr. Myint Myint Soe, SRD, Epidemiology

Japanese side

Prof. Y. Hamashima

Prof. T. Shikata

JICA

Mr. Shinoura

Mr. Takashima

8月14日（水）

9：00　医学研究局で打合せ。

10：00　新ラングーン総合病院訪問。

管理事務長のDr. U Tin Aung Htunと羽根田先生の出迎えを受ける。その後の新病院運営の実体を視察。

10：20　Healthへ直行。Dr. U Kyaw所長と面談。肝炎の疫学報告を聞く。

14：00　ウ　サン　ユ大統領，濱島教授を招き懇談したいとのことで大統領迎えの車で大統領官邸へ。U Tun Wai保健大臣と3人で，先ず大統領から，Chairman U Ne Winが濱島に従来のビルマへの医療協力貢献について親しくお礼を述べるようにとのこと。

そのメッセージを大統領から承る。

8月15日（木）

6：45　Meeting

9：00　保健局長Dr. U Tin Oo,濱島教授に会いたいとのこと。土砂降りの中をHealth

Dpt.へ行く。ＪＩＣＡより依頼の

①１９８５年度消化器病プロジェクトに対する供与機材リストの追加について，ビルマ側よりの正式要請リストの作り直しについて。これを直ちに了承。

②１９８５年度ビルマ側カウンターパーツ４名全員のA2・３Formを早く提出すること。

③１２月と３月に来緬する京都大学からの日本人専門家Ａ１Formを大至急出すよう。

以上の案件につき，その場で出してくれた。（これらのcopyはすべて持ち帰った。）

１０：００　医学教育局Pe Thein局長が濱島に会いたい旨連絡あり。すぐに医学教育局に飛ぶ。ＪＩＣＡ受入れ予定のビルマ医学教育視察団３名のＡ２・３Formを直ちに日本大使館に提出するよう要請。立ち処にＯＫで２，３日内に出すことの約束をとりつけた。

１３：１０　医学研究局においてR／D草案ならびに新プロジェクトに対する研究実施方針の討議をする。

１５：００　ォン　タン　バツー局長，ミミ　キン副所長と，濱島，志方教授４名によって約３時間にわたり今回討議した内容のまとめ"Memorandum"の原稿打合せ。

（Memorandumは別に記載。）

８月１６日（金）

８：３０　志方，濱島，Dr. Hla Myint.動物センターのU　Khin Maung Zaw４人で将来の肝炎感染実験室の計画打合せ。独立した建物のプランを検討する。

１１：３０　日本大使館に赴き塚本大使に，サン　ユ大統領との面談内容の報告をする。

８月１７日（土）　志方教授ペグー訪問（羽根田医務官夫妻案内）

８月１８日（日）

6時４５分発で，ラングーン空港を出発。パガンに９時２０分着。パガン郊外の部落訪問。住民の飲料水，食物などの調査。泥水のイラワジ河の水をそのまま飲料水として使用しているので驚く。

８月１９日（月）

８：４５　パガン空港発。

９：１５　マンダレー着。マンダレー医学校へ直行。１０時より医師と学生に講義。学長のDr. Tin Tun，院長のDr. Khin Maung Thanの出迎えを受け，講義は内科のDr. Khin Maung Win教授の座長で濱島教授より先に，続いて志方教授が夫々約１時間づつ講演をした。濱島教授のタイトルは"Cellular engineering in autoimmune dieseases"，志方教授のは"Hapatitis and hepatoma"。

講演終了後，２００人を超す聴衆から万雷の拍手を得，医師，学生から数多くの質問を受けた。マンダレーの方がラングーンよりも活気に満ちている印象を強く受けた。

午後，マンダレー西南部の大きなバザーの見学，原地人の食生活ならびに衛生状態，肝炎多発地区の給水，汚水処理などの実状をつぶさに視察した。

8月20日（火）

午前6時起床。７：００よりマンダレー西方約４０キロ離れたサガイン地区を訪問する。この地はかつての日本軍のおびただしい数の戦死者を出した激戦地であるが，またウィルス性肝炎多発地区でもある。貧しい部落民の実生活を詳細に調査した。

１３：００より医学校病理学教室のスタッフと面談。マンダレー地区の最近の重要な疾患の特徴について討議する。

その時の出席者は

マンダレー病院病理コンサルタント

Dr. Khin Maung Yin

Dr. Hla Min

医学校病理学教室

Dr. Hla Oo 教授

Dr. Saw Mya Yee

Dr. Khin Aye Kyi

8月21日

１０：００　保健大臣，３人の局長（医学研究局，保健局，医学教育局）と濱島，志方両教授。膝を交えての懇談。約1時間半に及び，数々のビルマの医療協力について意見の交換をする。その席上，U Tun Wai 保健大臣から直接に述べられた項目は以下のとおり，

①ＤＭＲの新プロジェクトに関するＲ／Ｄの最終正式案をば，東京（ＪＩＣＡ）から速やかにビルマ政府の方にご提出願い度い。

②京大での１９８５年度新しい４名の研修員を１０月頃に日本に送りたいのでよろしく。その４名のＡ２・３Form全部はもう準備が出来た。

③日本政府受入れの医学教育視察団３名はマンダレー医学校長Prof. U Tun Tin，医学教育局所長Prof. Daw May May Yi，第一医学校生理学教室Dr. Thin Thin Hlaing が選ばれ，その公式Ａ２・３ Formは調査員の離緬時までに渡すことが出来る。

④新病院に昭和６０年（１９８５）１２月から来る日本人専門家３名，および昭和６１年（1986）３月に来る日本人専門家４名，計７名のＡ１ Formはすでに正式に日本大使館の方へ送付した。

⑤マンダレー病院の公式要請はすでに日本側にお願いしてあるが，是非その実現をみたい。と云うのは，すでに医学教育の新方針にもとづいて，３年後にはマンダレーに新しい教育病院が必要で，もし実現されない場合には計画を急ぎ変更しなければならない。

マンダレー病院の件に関しては濱島は，日本側から何も正式な話を伺ってないので今の時点では何も申し上げるものはないと答えておいた。

１５：００　ＤＭＲの講堂で志方教授の肝炎ウィルスに関する特別講義で，医学研究所内のスタッフ一同大いに感激。あとでオン　タン　バッー局長が濱島に素晴しい人を連れて来て頂いて有難うと感謝してくれた。

８月２２日（木）

１０：００　第一医学校で学生に講義。

先に濱島が＂Stem cell replacement in autoimmune diseases＂と題して若年性糖尿病モデルマウスに骨髄の幹細胞を健康なものに置き換えてやると，病変が顕著に減少した旨の講演をしたところ，多くの質問が出て大変勉強になったと目を輝やかして聴き入っていた。次に志方教授が＂Hepatitis and hepatoma＂の講演，多くの質問が出た。

８月２３日（金）

８：３０　オン　タン　バッー局長との＂Memorandum＂の最後の詰め。

１１：００　日本大使館に塚本大使を訪ね，今回訪緬調査団の経過報告を行った。

１４：００　ＤＭＲでの特別講義。

＂Cellular engineering in autoimmune diseases＂と題して，ＭＲＬ／lpr，Ｂ／ＷＦ１　hybrid，ＢＸＳＢ，各モデルマウスの骨髄移植実験による最近の研究成果を講義。日本人研究者が来る毎に斬新な研究データを示し，著しい進展を示していることに驚いていたようである。

Ⅲ－３　総　括

ビルマのDepartment of Medical Researchに於けるウィルス性肝炎のプロジェクトについて

1. ビルマに於けるウィルス性肝炎の現状

ビルマに於ける衛生環境は非常に悪く，未だマラリヤ，デング熱，消化管感染症などまん延しており，現在の状況からはこれらの感染症が近い将来撲滅されるとは考えにくい。

感染症の中でウィルス性肝炎は近隣のタイ，インドなどと比較してもそれ以上に広がっているものと思われる。

Ａ型肝炎の年令別抗体保有率は１２才迄に９０％以上に達するが，Ａ型肝炎は子供の時感染す

ると極めて軽く経過するのでビルマ人にとっては重要な感染症とは考えられない。これはむしろ成人してからビルマに来る在留邦人にとつて重要な疾患である。

B型肝炎ウィルスのキャリヤー率は２０才代でピークの１５％に達するが，平均すれば１０％程度と考えられる。これは中国，タイなどとほゞ同様であり，インドよりもはるかに高い率である。その割には肝硬変，肝癌があまり重要視されていないのは，他の感染症が多いためであろう。デルタウィルスの感染に関しては全くデーターがない。

輸血後の非A非B型肝炎もあるといっているがはっきりしない。輸血の際にHBs抗原のチェックも行われていないのが現状だからである。

そこで問題はepidemic typeの非A非B型肝炎である。ビルマに於けるA，B，非A非Bの比率は流行の年によっても異なるが非A非Bが６０～７０％占めているのは間違いない。この肝炎は世界でもビルマ，インド，アフガニスタンなどに限局しておりビルマ自身が解決の道を歩まねばならない事は確かである。

2. epidemicタイプの非A非B型肝炎の研究方針

すべて流行例の臨床的，疫学的報告はインドなどでなされており，又マカカ属のサル，マモセットでの実験，２５㎜のウィルス様粒子などの報告もある。

これ迄のB型，A型などの研究の経験から次の様な方針が考えられる。

1) 糞便材料からのＩＥＭによるウィルス粒子の検出。

2) サルを使用しての上記の半精製ウィルス粒子の感染性の確認。

3) 糞便或いは肝臓からのウィルスの精製。

4) ポリクローナル或いはモノクローナル抗体の作製。

5) 抗原抗体のアッセイ系の確立。

6) 人及びサルの肝臓での免疫病理学的，電顕的ウィルスの証明。

7) 培養細胞を使用してのウィルスの増殖。

8) ウィルス核酸のクローニング

である。上記の仕事の遂行には材料の蒐集が先づ重要なポイントになる。材料としては1)感染初期の糞便，2)回復期血清（極期とペアで）3)肝臓の生検及び剖検材料　これらの材料の蒐集如何が一つの鍵をにぎっている。

3. ＪＩＣＡのプロジェクトとしてＤＭＲでの作業

先づDMRの中でどのdivisionがどの仕事の責任を持つか明らかにしておくのが重要である。

Experimental Medicine Division：

糞便からのＩＥＭによるスクリーニング　ウィルスの精製，糞便，血清，肝生検の蒐集。

Laboratory Animal Services Division：サルのメンテナンス

Pathology Research Division：サルの感染実験，肝臓の免疫病理学的，電顕的研究

Virology Research Division：ウィルスの精製，培養

Immunology Research Division：アッセイ系の確立

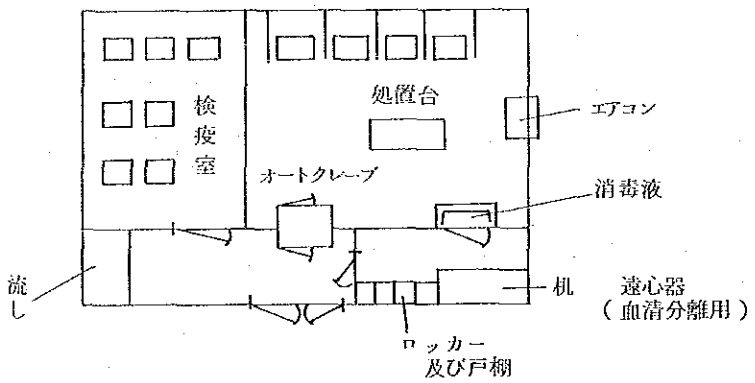
4. 昭和６１年度の実施計画

次に前述の研究方針の各項目は一時にスタート出来るものではない，一つの事実が確認されなければ次の項目に進めない。

先づスタートすべきものは材料の蒐集である。糞便はかなりの量になり更に－８０℃のフリーザーが必要かも知れない。

次のステップはＩＥＭによる糞便中のウィルスの確認とサルを使用しての感染実験である。ＩＥＭについては新しい設備は必要としない。

感染実験についてはBalayanがすでに述べているようにチンパンジー，マモセットのみならず，リーサスモンキイがこのウィルスに感受性を持っているのでビルマは最もこの実験に適した地域といえる。実験施設はＤＭＲに現在ないが物置きを改良することにより比較的容易に作り得る。屋根及び窓，入口の改修，部屋のしきり，オートクレーブ，洗い場，エアコンの設置，ケージ（すでに１０個あり）の追加が必要である。



尚，この図ではＰ３レベルのバイオハザードは必ずしも必要でなくＰ２レベルで十分と思われる。

従って，ここで必要なものは

1） 部屋の改修

2） オートクレーブ（両開き）

3） エアコン

4） 遠心器（血清分離用）

5） ロッカー及び戸棚

6） その他若干

次年度ウィルス抗原の精製の為には若干他の設備が必要となるが，免疫病理学的，電顕的研究の為には十分な設備があると考えられる。

尚材料蒐集に際して非A非B型肝炎の診断のためA型，B型，デルタウィルス感染を否定するためのA，B，δの診断のためのＲＩＡキットが必要である。上記の研究材料を蒐集するにあたっては必ずしもすべての肝炎患者が型別の診断が出来る必要はない。正確な診断が出来た少数例があれば十分である。ただこの国における現状を考える時，多くの黄疸患者が十分な肝機能検査をなされる事なく肝炎と診断されておりこの中にはマラリヤ，アメーバ膿病，胆管炎，閉塞性黄疸（アスカリスの迷入，癌など）多くの疾患が含まれている。先づ肝機能検査によつてウィルス性肝炎の診断を可能にする事が先決であり，次いでウィルスの型別の診断が出来るようにするのが順序であろう。肝機能検査は黄疸患者の一部に対して入院時１回だけ行われているに過ぎない。ＧＯＴ，ＧＰＴのアッセイキットを供与しても限りがありこれを持続的に供給する事が可能とは思えない。国全体の経済的レベルが上がるほかないがそれも期待出来るとも思えない。然し少くとも肝炎のプロジェクトの対象となる症例の診断の為にはトランスアミナーゼ測定のキットを供給すべきであろう。

Ⅳ MEMORANDUM

MEMORANDUM

DMR/JICA DISCUSSIONS ON THE PROJECT "RESEARCH ON TREATMENT OF INFFECTIOUS DISEASES OF THE ALIMENTARY SYSTEM" held between 12-24, August 1985.

The Preliminary Survey Team headed by Professor Y. Hamashima accompanied by Professor T. Shikata arrived in Rangoon to further discuss the DMR Research Proposal. DMR had previously presented the proposal to the 1st Preliminary Survey Team on 18-28 February, 1985 and an understanding was reached on various aspects of it as contained in the memorandum of 27 February 1985. In the light of the previous discussion in February and with a view to more detailed presentation regarding Hepatitis Research to Professor Shikata a modified version was again presented to JICA Team by DMR on 13 August 1985. The group discussion were attended by Director-General, DMR and Burmese Scientists who would be undertaking cooperative research for the above mentioned project and by Professor Hamashima, Professor Shikata and by Mr. Shinoura and Mr. Takashima, JICA Representatives (on the 13 August only).

Discussions were carried out on the basis of the modified version of the proposal and focused on the major research area of non-A, non-B hepatitis viruses.

Outcome of the discussion

1. It was agreed that the Major research areas would be as follows:

a) Research on non-A, non-B virus(es) and non-A, non-B hepatitis and its sequelae.

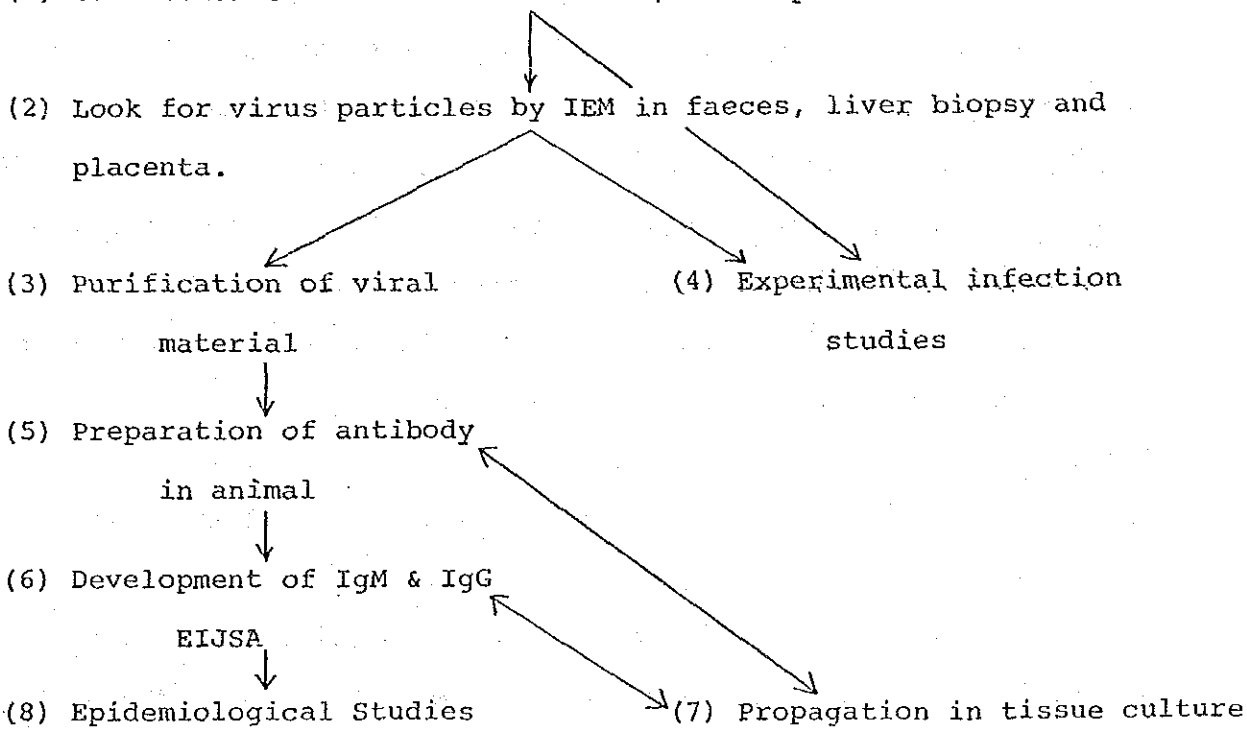
b) Research on Rota virus and other diarrhoea-associated virus and infections caused by these agents.

c) Research on Entomoeba histolytica and Amoebic infections of the gut and liver.

2. The research objectives A, B, C and D for Research on non-A, non-B viruses, non-A, non-B hepatitis and its sequelae was agreed upon. It was also agreed that the main emphasis of non-A, non-B research would be on faecal oral type.

Research objective A, B and D were extensively discussed and the following schematic work plan for undertaking the research was agreed.

Schematic Work Plan



As a result of the discussion it seems possible that animal infection studies could be carried out at DMR using Rhesus monkeys. DG agreed to provide the building for monkeys. Suitable safety measured will have to be instituted. However it may still be necessary to carry out some of the animal experimental infection studies in Japan. Professor Shikata will bring more detailed work plan for the above work in December.

3. During discussion about objective C the role of host versus virus factors were extensively discussed. Discussion did not progress to the stage of development of an acceptable and suitable approach to the problem. It was felt that more definite plans need to be developed as to how this objective would be approached. Japanese and Burmese Scientists will need time to develope a work plan.
4. Importance of the availability of research material such as serum, liver biopsy and autopsy specimen was stressed by Professor Shikata and other scientists and the preparation for such collections and initiation of the studies should be made early in time for the 1986 hepatitis season begining May.
5. Plan for Research on Rotavirus and their infections and other diarrhoea associated viruses and their infections was agreed upon as presented in modified version.
6. The modified version of Research on Entamoeba histolytica and amoebic infection of the liver and gut was presented and agreed upon.
7. As agreed upon in the Memorandum of 27 February 1985, it was again stressed by DG and generally agreed that recombinant DNA technology should be introduced to DMR under this project. The entry point

for introduction of DNA technology as presented by DMR seems ambitious. Professor Shikata suggested that DMR may try inserting the DNA gene encoding for the HBV into plasmids of E. coli as entry point for introduction of the recombinant DNA technology at DMR. He mentioned that the HBV gene may be available.

8. Regarding development of Immunopathology and Immunochemistry in the Project, the need and the way in which it will be developed has been included in the DMR Research Proposal. This was generally agreed. Special mention was made about detection of circulating immune complexes and viral antigen localization by means of peroxidase labelled antibody technique and if possible by employing the monoclonal antibodies. Professor Hamashima expressed the view that development in these areas will take place gradually.

9. The JICA Team expressed their view that some of the Research leads which emerge from the previous DMR/JICA technical cooperation project (Research on Major Arboviral Disease, Bacterial Enteric Diseases and the Application of its results for the Control of these diseases which terminated in April 1984) should be followed up during the present project especially those covering.

a) Research on Bacteriology of acute diarrhoea

b) Research on Dengue Haemorrhagic Fever

This was generally agreed and DMR had already presented (Annex of the Research Proposal) some of the Research leads on Bacteriology that will be followed up. Research leads to be followd up for Dengue will be developed by DMR Scientists and discussed with Japanese Scientist later.

LIST OF PARTICIPANTS

Preliminary Survey Team

1. Dr. Y. Hamashima .....Team Leader
2. Dr. T. Shikata

JICA Representatives

1. Mr. T. Shinoura
2. Mr. T. Takashima

DMR Scientists

1. Dr. Aung Than Batu .....Chairman
2. Dr. Mi Mi Khin
3. Dr. Than Than .....Pathology Research Division
4. Dr. Mg Mg Oo .....
5. Dr. Soe Soe Aye .....
6. Dr. Tun Pe .....Immunology Research Division
7. Dr. Hla Pe .....Biochemistry Research Division
8. Dr. Soe Thein .....Virology Research Division
9. Dr. Kyaw Moe .....
10. Dr. Hla Myint .....Experimental Medicine Division
11. Dr. Khin Mg Win .....
12. U Tun Khin .....
13. Dr. Tin Aye .....Bacteriology Research Division
14. Daw Mar Mar Nyein .....
15. Dr. Phyu Phyu Win .....
16. Dr. Myint Lwin .....Parasitology Research Division
17. Daw Than Saw .....
18. Dr. Myint Myint Soe .....Epidemiology Research Division

資　　料

資料 - 1.

TECHNICAL COOPERATION BETWEEN DEPARTMENT OF MEDICAL RESEARCH

AND

JAPAN INTERNATIONAL COOPERATION AGENCY

ON

RESEARCH ON TREATMENT OF INFECTIOUS DISEASES OF THE ALIMENTARY SYSTEM

DMR PROPOSALS FOR DISCUSSION WITH JICA PRELIMINARY SURVEY TEAM

1ST ------ FEBRUARY 18-28, 1985

2ND ------ AUGUST 12-24, 1985

C O N T E N T S

1. INTRODUCTION

The Biomedical Research Centre was donated by the Japanese Government and completed in 1980.

It comprises a laboratory building with laboratories for the Bacteriology Research Division, Virology Research Division, Pathology Research Division, Immunology Research Division, Physiology Research Division, Biochemistry Research Division as well as a Laboratory Animal House, and Library building and Transformer and Generator building. All buildings and laboratories were fully equipped.

Following completion of the Biomedical Research Centre, technical cooperation on " Research into Bacterial Enteric Diseases and Major Arbo-viral Diseases Project " was carried out by JICA and DMR. This project was started in 1980 and completed and terminated April 1984.

The Japanese Evaluation Team as well as the Coordination Committee noted that the Project had been satisfactorily completed. They further stated that it was essential for DMR to maintain its research momentum that further technical cooperation between DMR and JICA should be carried out in a new project between Japanese and Burmese scientists would have an opportunity for further collaboration.

The Burmese authorities made a formal request to the Japanese authorities late in 1984 for a new technical cooperation project between JICA and DMR entitled " Research on Treatment of Infectious Diseases of the Alimentary System ". The project outline initially formulated by DMR and forwarded to the Japanese side is given in Annex.

2. SELECTION OF RESEARCH AREAS

The following criteria will be used for selecting programmes for DMR/JICA cooperation:

1. Magnitude and priority as a health problem
2. Probability of finding a solution or an important clarification
3. Benifits expected from the application of the results of successful research effort
4. The potential usefulness of the results of the research in finding solutions to other problems
5. The level of ongoing research on the problem both in Burma and Japan
6. Suitability of the problem for cooperation.

3. BRIEF DESCRIPTION OF THE DEPARTMENT OF MEDICAL RESEARCH

The Department of Medical Research has the following aims:

1. Improvement of the health of the people of Burma
2. Improvement of the economy of the country
3. Contribution towards scientific knowledge
4. The rapid application of new and emerging knowledge towards solution of health problems

The mission of the Department of Medical Research

1. To conduct biomedical research
2. To promote, support, organize and coordinate all biomedical research in the country
3. To provide the infrastructure necessary for effective biomedical research
4. To provide training in medical research

The Department of Medical Research is organized into 15 Research Divisions and 3 Research Units and 5 support Divisions as follows:

Research Divisions

1. Bacteriology Research Division
2. Biochemistry Research Division
3. Clinical Research Division
4. Epidemiology Research Division
5. Experimental Medicine Division
6. Immunology Research Division
7. Medical Entomology Research Division
8. Medical Statistics Research Division
9. Nuclear Medicine Research Division
10. Nutrition Research Division
11. Parasitology Research Division
12. Pathology Research Division
13. Pharmacology Research Division
14. Physiology Research Division
15. Virology Research Division

Research Units

1. Clinical Research Unit for Snake-bite
2. Clinical Research Unit for Cerebral Malaria
3. Clinical Research Unit for Indigenous Drug

Support Divisions

1. Administration Division
2. Central Biomedical Library
3. Instrumentation Division

4. Laboratory Animal Services

5. Publication Division

The total staff strength of the DMR is 315 of which 108 are administrative personnel. There are 81 scientists of which 39 are medical doctors, and there are 126 technicians. Among the scientists and technicians there are 6 Ph.D., 49 with post-graduate qualifications such as M.Med.Sc., M.Sc., Diploma in Bacteriology, etc. In addition the DMR employs on a contractual basis temporary staff (both medical doctors and technicians) for various Research Projects supported by International organization like WHO, UNICEF and the number range from 100 to 200 a year.

The DMR also gives research training and research supervisions to M.Sc. students number from 10 to 20 a year.

The Research and Support Divisions are accommodated in the

-Biomedical Research Centre Laboratory Building

-Clinical Research Centre Building

-The Social Medical Research Centre Building

-Central Biomedical Library

-Laboratory Animal House

Clinical Research Units are located in Hospitals where patients are being studied. Thus, the Clinical Research Unit for Cerebral Malaria and the Clinical Research Unit for Snake-bite are both at the Tharawaddy Hospital while the Clinical Research Unit for Indigeous Medicine work at Indigeous Hospital.

Many of the Research Division have field teams while work for extended periods of one month or more in different locations

throughout Burma. Thus the Parasitology, Entomology and Epidemiology Research Divisions work for periods in the foothills of the Pegu Yoma on Malaria Field Research, and the Epidemiology Research Division and Parasitology Research Division work in villages for Ascariasis Research, and the Nutrition Research Division work in the hilly regions on Goitre Research.

4. AIMS OF THE PROJECT

The Project aims to conduct research on viral and parasitic infections of the liver and gut. The goal of the comprehensive studies is to achieve improvements in diagnosis, control and treatment of diseases due to these infections in Burma.

The disease-problems to be studies include:
Non-A, Non-B hepatitis and its sequelae, amoebic infections of the liver and gut and their sequelae, Rotavirus and other diarrhoea-associated viral infections of the gut and their sequelae.

An equally important aim is to strengthen research capability particularly in the basic disciplines such as immunopathology and immunochemistry and in newer approaches such as Recombinant DNA technology so as to provide a sound foundation for present and future research efforts.

Research Areas are as follows:

1. Research on NANB virus(es) and NANB hepatitis and its sequelae.
2. Research on Primary hepatoma.
3. Research on Rotavirus and other diarrhoea-associated viruses and infections caused by these agents.

4. Research on Entamoeba histolytica and amoebic infections of the gut and liver.

5. Research on Bacteriology of acute diarrhoea.

5. RESEARCH ON NON-A, NON-B VIRUS(ES) AND NON-A, NON-B HEPATITIS

Research objectives/activities

A. The identification, isolation and characterization of faecal/oral NANB virus present in Burma.

B. Development of methods for determining infection with faecal/oral NANB viruses.

C. Immunopathological, pathophysiological and clinical investigation of NANB hepatitis - including hepatic failure in pregnancy with NANB hepatitis.

D. To produce NANB hepatitis in experimental animals with the putative virus, and to do transmission studies.

A. The identification, isolation and characterization of faecal/oral NANB virus present in Burma

(i) To identify, isolate, purify and characterize putative non-A, non-B virus.

Relevant Background

Outbreak of viral hepatitis that occurred in Burma are referred to as epidemic or faecal/oral non-A, non-B hepatitis (Hla Myint et al., in press) to differentiate them from post transfusion non-A, non-B hepatitis which is widespread throughout the world.

By employing immune electron microscopy (IEM) Balayan et al. (1983), Sreenivasan et al., (1984) and Kane et al., (1984) respectively reported visualization of 27-30 nm size

virus-like particles in the stools of an acute non-A, non-B patient. Similar virus-like particles of 27 nm in diameter was visualized in 4 acute phase stool specimens of patients in an epidemic of non-A, non-B hepatitis in Rangoon in 1982 (Dr. Hla Myint, DMR).

The existence of at least two transmissible agents of post transfusion non-A, non-B hepatitis has been documented in recent studies by Shimizu et al, (1979), Bradley et al (1979; 1983) and Yoshizawa et al, (1980) in experimentally infected chimpanzees. These particles banded at a density of 1.30-1.31 Gm/cc in cecium chloride gradients (Bradley et al, 1979).

Thus, approaches used in the past to identify hepatitis A and B antigen have been applied to the search for non-A, non-B antigens. However, these approaches have fallen short of fulfiling acceptable serologic criteria for a specific association with non-A, non-B hepatitis (Dienstag, 1983).

After a decade of study, our knowledge of viruses causing non-A, non-B hepatitis is strangely sketchy. Distinct agents appear to be responsible for epidemic (or) faecal/oral type and post transfusion type of non-A, non-B hepatitis. Although many types of virus-like particles and many candidate antigens have been reported, we still lack conclusive evidence associating these with a non-A, non-B virus. (Prince, 1983).

Work Plan

1. Visualization of virus-like particles in the acute-phase stool specimens of non-A, non-B hepatitis by IEM.
2. Biophysical characterization of faecal/oral non-A, non-B virus recovered from stools:
   (a) Buoyant density
   (b) Sedimentation coefficient
3. Purification of F/O non-A, non-B virus from stool. (Established procedure).

(ii) Propagation of NANB virus in cell line cultures:

Non-A, Non-B agents have not been isolated or serially propagated in tissue culture.

The in vitro cultivation of HAV eluded all attempts for several decades. However, HAV has fully yielded to efforts to grow in cell culture, and most importantly, it can be grown in cells that are suitable for vaccine development (1, 2).

The virus was repeatedly propagated in primary explant cell cultures of marmoset liver and in the normal foetus heas kidney cell line (FRh k6). Identity of the virus was established by immunofluorescence, radioimmunoassay, IEM, and marmoset innoculation.

Recently, also, a model system for the study of HBs Ag has become available in the form of human hepatoma cell lines which produce this antigen. Hep 3B has also been reported to be a cell line that produces HBs Ag (3, 4).

Liver tumor cell lines derived from HBV-infected people (3, 5-8), and cells transferred with HBV DNA (9). However those

cultures were not capable of making the whole virus or to release the HBV into the culture supernatant. Most produced only HBs Ag although some transfested cells appeared to make virion core antigen (HBC Ag) for limited periods.

Several cultures have been established from: patients with primary liver cancer PLC/PRF/5 and Hep 3B which appears to contain the HBV genome and to make large quantities of HBs Ag (3 & 7). Further studies have shown that cultured human lymphoblastoid cells RAC/BM culture could possibly produce infection with HBV (9).

Since the above studies have provided proof of a positive indication it would appear to be feasible in elucidating and exploiting the attempts of propagation of the non-A, and non-B hepatitis virus recovered from epidemic situations.

Thus, it seems that attempts for the isolation of non-A, non-B agents in tissue culture systems are worthwhile attempting.

Work plan

Putative viruses detected by IEM in the stools of non-A, non-B hepatitis cases will be utilized. Attempts will be made to grow these putative viruses in candidate cell culture system (primary cell culture systems as well as cell line systems). These include cell culture systems that has been found suitable for cultivation of HAV and HBV such as: primary AGMK cell.

Attempts to propagate established continuous cell lines such as PLC/PRF/S, Hep.3B, FRhK6, FRhK4 & Vero which are available from commercial sources.

During this preliminary stage using continuous established cell cultures, various basic factors such as incubation tempera-

tures, adsorption, treatment of cells, holding time for incubation should be standardized to provide a maximum output of infectious virion.

Source of material would be stools and serum obtained during the acute onset of infection from non-A, non-B hepatitis after exclusion of HAV and HAB.

For the preparation 1° cell culture, normal hepatocytes, Kupffer cells (liver macrophages) and cell population of haematopoietic origin and placental tissues will be used.

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(iii) Cloning and expression of putative NANB genes in E. coli

(see under 9 (b))

B. Development of methods for determining infection with faecal/oral NANB viruses

(i) To find serological markers of NANB hepatitis

(ii) To demonstrate significant antibody titer against the putative virus in patients with NANB hepatitis

(i) To find serological markers of NANB hepatitis:

Relevant background

The diagnosis of non-A, non-B hepatitis is based on the exclusion of viral hepatitis A or B.

Many investigators have developed tests with agar gel diffusion or counter-electrophoresis that appears to show specific antigens or antibodies for non-A, non-B hepatitis. These tests are controversial, and there is no general agreement that precipitin lines are specific for non-A, non-B hepatitis or that different laboratories observe the same reactant (Gitnick, 1984).

They used sera from acute-phase patients as a source of "antigen" and sera from convalescent patients as a source of "antibody". Shirachi et al., (1978) initiated the detection of

non-A, non-B antigen or antibody either by agar gel diffusion or by counter-electrophoresis techniques. Suh and colleagues (1981) investigated the specificity of a precipitin system and found that the reaction was not a typical antigen-antibody reaction. Other researchers Chircu et al. (1980), Hopkins et al. (1981), Mori et al. (1981) and Gitnick et al. (1982) contend that the immune precipitation reactions are real, but are present only in few patients.

If one or more of the immuno-precipitin test turns out to be reproducible and specific for non-A, non-B hepatitis, it will be an important base line for developing more sensitive tests. Although several techniques are currently being evaluated in research laboratories, no specific serologic test is available as yet for the diagnosis of NANB hepatitis. Serologic antigen-antibody systems have not proved to be repeatable or specific (Gitnick, 1984).

Work Plan

1. Source of antigen
   (a) Virus-like particles will be isolated from acute-phase stools of non-A, non-B hepatitis (positive by IEM) and purified.
   (b) Placenta of pregnant woman died of non-A, non-B hepatitis will be collected, homogenates will be screened for virus-like particles by IEM and these particles will be isolated and purified.
2. Source of antibody
   (a) Antibody from convalescant sera of known non-A, non-B patients.

(b) Production of antibody in laboratory animals --- such as guinea pigs, rabbit -- by using antigens from above 1.

3. Testing of antigen-antibody reaction by agar gel diffusion and counter-electrophoresis techniques.
4. Application of the test in the diagnosis of non-A, non-B hepatitis patients.

(ii) To demonstrate significant antibody titre against the putative virus in patients with NANB hepatitis.

Relevant Background

The immunological tests developed by many investigators are controversial, and there is no general agreement that precipitation lines are specific for NANB hepatitis or that different laboratories observe the same reactant (Gitnick, 1984).

Dienstag et al.(1979) have reported an observation that high levels of circulating immune complexes in NANB patients.

The "antigen" (from acute phase sera) had many properties of immune complex. The "antibody" (from convalescent sera) was best described as a variety of complement breakdown products (Gitnick, 1984).

Under these circumstances, an alternative approach from the above (B - (i) is worthwhile. By employing immune electron microscopy (IEM) and using putative virus particles as antigen an attempt will be made to detect the rising antibody titre in acute NANB patients.

Work Plan

1. Virus-like particles (VLPs), detected by IEM of acute-phase stools of NANB patients, will be isolated and purified.

2. Paired sera from acute NANB patient will be collected to determine antibody against VLPs.
3. IEM examination will be carried out with different dilutions of patients sera against VLPs to determine the rising titre.

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C. Immunopathological, pathophysiological and clinical investigation of NANB hepatitis ---- including hepatic failure in pregnancy with NANB hepatitis

Relevant Background

In the recent epidemic of epidemic NANB hepatitis in Rangoon in 1982, the highest case fatality rate was observed in pregnant women with NANB hepatitis as compared to hepatitis in non-pregnant females or males, or when compared to hepatitis caused by A or B virus. Increased severity and fatality among pregnant wemen was also reported in an epidemic of NANG hepatitis in Mandalay in 1976. Similar findings with high mortality in pregnancy were reported by Kane et al. (1984), Khuroo et al.(1981), Borhanmanesh et al., (1973) and Naidu et al.(1957).

This feature of conspicuously high mortality among pregnant females with NANB hepatitis in contrast to non-pregnant females, males and infection with hepatitis B and A is noteworthy. It is of great importance to know the reason for this obvious difference and whether NANB virus is more virulent in pregnant women or whether immune responses are different in the case of NANB infection in pregnancy.

The differences in clinical course and prognosis in fulminant hepatitis A, B and NANB may be a reflection of differences in the mechanisms underlying the initiation and progression of the hepatic necrosis, as well as the potential for hepatic regeneration upon which survival ultimately depends. In cases of fulminant hepatitis B, Gimson et al. (1981) have shown a more rapid clearance of HBV antigens than in uncomplicated cases, because of an enhanced antibody response. This could be the basis for

subsequent immune complex deposition in the liver sinusoids with ischaemic necrosis of hepatocytes as a result, a hypothesis for which there is also experimental evidence (Mori et al, 1981). In contrast, the evidence suggests that the HAV is directly cytopathic (Bradley et al, 1978) and the hepatic necrosis that occurs in those patients with fulminant course could be the consequence of a larger inoculum of the virus or an impaired antibody response. The process underlying the liver damage in fulminant NANB hepatitis would appear to be a slower process, as judged from the longer period before signs of encephalopathy appear (Gimson et al, 1983). Of crucial importance in the recovery from fulminant hepatic failure is the rate of hepatic regeneration, but this is difficult to assess, and whether there are differences between the three types of viral hepatitis is not known.

In their study of post transfusion hepatitis, Dienstag et al (1979) reported an observation that high levels of circulating immune complexes in non-A, non-B patients, and they suggested that these immune complexes may contain a virus specific antigen. Again, Ohori et al (1981) suggested that the immune complex containing an 80K protein may be an antigenic reactant of non-A, non-B hepatitis.

The "antigen" (from acute phase sera) had many properties of immune complex. The "antibody" (from convalescent sera) was best described as a variety of complement breakdown products (Gitnick, 1984).

Work Plan

1. Monitoring of the immune status (humoral and cellular immunity)

of patients with viral hepatitis A, B and NANB, especially in pregnant women.

2. Detection of circulating immune complexes in faecal/oral NANB hepatitis.
3. Morphological characterization of pathognomonic features of NANB hepatitis by light microscopy and Electron Microscopy.
4. Assessment of liver cell function recovery:

   measurement of --- serum AFP
   --- bile acid conjugation rate
   --- fibrinogen turnover rate
   --- albumin turnover rate

5. Determination of biochemical factors in fulminant hepatic failure (FHF)

   --- utilization of glucose
   --- serum electrolytes

6. Clinical study of FHF with VHA, VHB and NANB

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D. To produce NANB hepatitis in experimental animals with the putative virus and to do transmission studies

Relevant background

Outbreaks of viral hepatitis that occured in Burma are referred to as epidemic or faecal/oral NANB hepatitis (Hla Myint, et al., in press). By employing immune electron microscopy (IEM)

Kane et al., 1984, Dr. Hla Myint (DMR) respectively reported visualization of 27 nm size virus-like particles in the stools of acute NANB hepatitis patients. To confirm that these particles are the causal agents of Burmese faecal oral NANB hepatitis, transmission of the disease to appropriate experimental animal is a necessity. Many workers have successfully transmitted NANB hepatitis to chimpanzee and marmosets either by injecting infected serum or using infected stool extracts. Variable morphologic changes were observed by different workers.

Work Plan

1. To produce NANB hepatitis in experimental animals by established method and to demonstrate the characteristics histological and other abnormal feature of these lesions.
2. To see whether infection with the putative virus isolated from Burmese NANB hepatitis patients produce characteristic lesions in experimental animals.
3. To characterize both ultrastructural and histological changes in human liver biopsies natually infected with NANB hepatitis.
4. Isolation of virus-like particles from stool, serum and liver homogenate of experimentally infected animals and to perform cross challenge studies on appropriate experimental animals.
5. To study the pathogenesis of NANB hepatitis both in the human cases and experimentally infected animals by appropriate methods.
6. To search for the tissue markers for NANB hepatitis e.g. gene probe technology, autoradiography.

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6. RESEARCH ON PRIMARY HEPATOMA

Research objectives/activities

A. Cloning of alpha foetoprotein gene.

B. Development of methods for early detection of primary liver cancer in man.

A. Cloning of alpha-foetoprotein gene (see 9(b))

Production of AFP (and anti-AFP) by recombinant technology is feasible and economical in the long run. It is an appropriate candidate for exercise in establishing DNA cloning techniques in DMR.

Work Plan

1. Establishment of recombinant DNA techniques
2. Development of diagnostic tests based on recombinant DNA technology including ELISA and RIA for AFP concentration and scanning of radio-labelled anti-AFP globulin.

B. Development of methods for early detection of primary liver cancer in man.

Concept about PLC have changed in recent years, such as: (i) HBV, aflatoxin and other chemicals rather than other factors are considered aetiologic factors in high risk areas, (ii) early diagnosis of PLC has progressed from symptomatic to subclinical

stages; combined analysis of serum AFP and serum alanine amino-transferase activity (ALT) rather than symptoms, signs, and complex biochemical findings are emphasized, (iii) in contrast to previous concepts, PLC can have relatively long natural history (Tang et al., 1982).

The diagnosis of PLC has progressed from "post-mortem diagnosis" in the 1930s through "clinical diagnosis" to "subclinical diagnosis" in the 1970s.

Although AFP remains the best marker for diagnosis of sub-clinical PLC, new tumour markers are required because one fifth of all liver cancers in high-risk areas and more in low-incidence areas do not have an elevation of AFP. But of all tumour markers, AFP remains the best and is most specific; other markers may aid in the diagnosis of AFP-negative PLC. Targeting and quantitative localization of hepatic carcinoma (AFP-positive) cells in tumour-mass using radio-labelled anti-AFP globulin sensor could be a novel way to detect a small and early primary hepatic carcinoma. Most probably, it could lead to a new strategy for the management of primary liver carcinoma, namely drug-targetting therapy.

Work Plan

1. An attempt will be made for early detection of AFP in the tumour mass, even before appearing in the serum of patients, by using radio-labelled anti-AFP globulin sensor.
2. Application of new tumour markers: ferritin, hepatoma-associated antigen, hepatoma-liver antigen, 5'-nucleotide phosphodiesterase isozyme V, gammaglutamyl transpeptidase isozyme, carcinoembryonic antigen.
3. To find potential markers by follow-up study of chronic cases.

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7. RESEARCH ON ROTAVIRUSES AND OTHER DIARRHOEA-ASSOCIATED VIRUSES AND THEIR INFECTIONS

Research objectives/activities

A. Defining and characterization of rotavirus, and other viruses associated with diarrhoea.

B. Study of environmental pollution by rotavirus and other enteric virus.

C. Pathophysiology of enteric virus infections.

A. Defining and characterization of rotavirus and other viruses associated with diarrhoea.

(i) Non-rotaviral agents in viral diarrhoeas.

Rotavirus has been well established as an important cause of acute diarrhoea in children. However, the role of other viruses in the causation of acute diarrhoea has not been well documented except for the Norwalk agent. A number of viruses have been implicated as causative agents of acute diarrhoea but most of the reports involve a small number of subjects, proper controls were not included and in some control studied these viruses were detected with equal frequency in both diarrhoea as well as in controls (1). Especially, the small round viruses have not been well studies and their role in acute diarrhoea needs to be defined.

It has been well established that rotavirus is the most important single pathogen implicated in diarrhoea among children under 3 years of age in this country. Although the aetiological role of rotavirus in childhood diarrhoea has been well defined, there are very few reports on the role of other viral agents in the causation of diarrhoea especially in Burma and other South East Asian countries. A preliminary study has already been in progress at the Department of Medical Research to search for viral agents other than rotavirus in diarrhoea employing immune electron microscopy.

Work Plan

Objectives

1. To determine the proportion of acute diarrhoea associated with non-rotaviral agents.
2. To study the clinical features of acute diarrhoeal cases associated with these agents
3. To determine the seasonal, age and sex distribution of acute diarrhoea cases associated with these agents.

The study will be undertaken in children under the age of five years attending hospital with a history of acute diarrhoea. The study will be over a period of two years. Age and sex matched controls who have not had diarrhoea within the past month will be included in the study. Stool specimens will be collected from the diarrhoea and controls and will be screened for the presence of rotavirus by ELISA. All stool samples will also be examined by immune electron microscopy (IEM) using pooled human immunoglobulin. Stool samples in which virus particles are detected by IEM will be

examined by electron microscopy to morphologically characterise the virus particles and also by IEM using specific antisera. The stool samples will also be tested for the presence of adenovirus by ELISA(3) and for the presence of calicivirus by RIA(2) depending upon the availability of these test systems.

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(iii) Avidin-Biotin Enzyme Immunoassay for the Diagnosis of Rotavirus Infection

Background

The rapid and accurate diagnosis of infectious diseases is essential both for the immediate care of the patient and for the introduction of necessary public health control measures. Enzyme immunoassays have a number of advantages which make them ideally suitable for use in diagnostic virology. These advantages include

sensitivity, objectivity and versatility. However the performance characteristics of solid phase immunoassays depend to a great extent upon the binding characteristics of the labelled immunoreactants utilized in the assay system. This is particularly true in EIA since the direct labelling of immunoglobulins with large molecules can lead to polymers of variable size and reactivity. Low molecular weight markers which can be linked to perform active enzyme conjugates have been used to overcome this problem. One of the efficient methods of accomplishing such linkage is to utilize biotin, a low molecular weight vitamin with a high affinity for avidin, a protein which can be efficiently labelled with enzyme or other marker molecules. Biotin can be linked to immunoglobulins by a number of methods resulting in stable monomeric biotin-immunoglobulin complexes. The amount of biotin labelled immunoglobulin reacting with antigen can be quantitated by the addition of avidin which is couples with biotinallated enzyme. Enzyme immunoassays utilizing biotinallated reagents are more convenient than the conventional enzyme immunoassays for large scale usage since the same labelled reagents (e.g. avidin-enzyme) can be utilized for all the immunoassays (Geusdon et al., 1979).

Work Plan

Objectives

To develop an avidin-biotin enzyme immunoassay for the detection of rotavirus in stools.

Methods

The test system will be designed as a solid-phase double antibody sandwich enzyme immunoassay. Microtitre plates will be coated with specific rotavirus antibody (IgG fraction) while the second

antibody will be biotin-labelled rotavirus antibody. Avidin conjugated to an enzyme (peroxidase or alkaline phosphatase) will be used as the detector system.

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B. Environmental pollution by enteric viral infections

Pollution of the environment by pathogens is an important factor to be considered in epidemiological studies on infectious diseases. Enteric viral pathogens are shed in very large numbers by infected persons and poor hygienic conditions will lead to environmental pollution by these viruses with subsequent spread of diseases. The occurrence and survival of the enteric viral pathogens in the environment need to be studies extensively in order to institute proper control measures to prevent disease transmission.

The occurrence and survival of organisms in the environment is an important factor to be considered in epidemiological studies of infectious diseases. The occurrence and medical significance of excreted viruses is a rapidly changing field of knowledge. Many viruses are known to be excreted faecally by humans. Typically they infect the alimentary tract with or without producing symptoms and are shed in very large numbers by infected persons. Diseases caused by these viruses range from the mild to the serious or even fatal. Viruses cannot multiply outside living cells, therefore, in the environment their numbers only decrease.

However, under favourable conditions like neutral pH, the presence of particulate or organic matter, moisture and in particular low temperatures, they can survive for prolonged periods of time.

The enteroviruses (chiefly, poliovirus, coxsackievirus, and echovirus) and to a lesser extent adenoviruses and reoviruses have been the only excreted viruses to be extensively studies in the environment. It was partly because certain other important excreted viruses, particularly hepatitis A and human rotavirus, until recently, cannot be grown in cell culture and so their infectivity cannot be easily studied. Now with advanced methodology, studies on the occurrence and survival of most of the faecally excreted viruses have become feasible.

Work Plan

Objectives

1. To detect enteroviruses, hepatitis viruses (A and NANB), rotavirus, enteric adenovirus and other diarrhoea viruses in water, sewage and soil.
2. To determine the seasonal prevalance of these viruses in the environment.
3. To determine the infectivity titres of some of these viruses in the environment.

Methods

Samples of water (from various sources), sewage and soil will be collected from different areas and at different times. The virus present in the samples will be concentrated by membrane filtration and will be cultured in cell lines as well as examined by electron microscopy. The viruses will be identified by the established methods and their infectivity titres in the samples will be assayed.

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8. RESEARCH ON ENTAMOEBA HISTOLYTICA AND AMOEBIC INFECTION OF THE LIVER AND GUT

Research obfectives/activities

Development of immunodiagnostic test for invasive and non-invasive amoebiasis.

Relevant Background

Dysentry dur to Entamoeba histolytica and hepatic amoebiasis are common disease in the country. Among the single leading causes of morbidity at the Rangoon General Hospital, amoebiasis is at the 7th position. The prevalence of this tropical disease has presented difficult diagnostic and management problems especially when the liver is involved.

It has also been known from the early days that E. histolytica consists of two morphological identical organisms and pathogenic and invasive and the other harmless commensal, (Bray & Harris, 1977). Among the available methods of biochemical taxonomy or biochemical characterization, the most successful and versatile technique is reported to be electrophoresis technique (Bullini, 1984). Strains differentiation on the basis of their characteristic isoenzyme patterns of Entamoeba histolytica and other amoebae inhabiting man's

gastrointestinal tract, using isoenzyme electrophoresis technique was demonstrated by Sargeaunt & Williams, (1979). Jackson & Gathram (1985) had shown the relationship between the seroepidemiological data with the zymodemes of E. histolytica. Gathram & Jackson (1985) had also shown the frequency distribution of E. histolytica zymodemes in rural South African population: Recently during an outbreak of Shigella. Entamoeba histolytica are also detected in stools of Shigella patients, and caused confusion in diagnosis. The importance of being able to distinguish between pathogenic and nonpathogenic train of E. histolytica has become more evident.
It is of special interest and importance for the country to investigate and to study the invasive and also of non-invasive amoebiasis.

Work Plan

Phase I - To establish axenic culture of amoeba in the Division.

Phase II(a) - To study the zymodeme pattern of E. histolytica by isoenzyme electrophoresis.

Phase II(b) -

As for detection of extraintestinal amoebiasis such as amoebic liver abcess (ALA) or other tissue stages, Enzyme linked immunosorbent assay (Agarwal et al., 1982, Tandon, 1981). Direct and indirect immunofluorescent techniques (Gilman et al., 1980) are demonstrated to be of value.

Phase II(b)

The following immunodiagnostic methods will be established

(a) Direct Haemagglutination Test

(b) Direct and Indirect Fluorescent Antibody Method

(c) Enzyme Linked Immunosorbent Assay and

(d) Others.

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9. RESEARCH CAPABILITY STRENGTHENING

Some of the activities undertaken under various Reserach Areas may be regarded as Research Strengthing activities.

However, it is felt that a deliberate strengthening effort should be made in certain areas, in order that a firm foundation be provided for the high level of research planned under the Project as well as for future high level research at DMR.

The particular areas identified for special strengthening of research and capability are:

A. Immunopathology and Immunochemistry

B. Recombinant DNA technology

C. Infrastructure support in general would also be necessary for the research planned under the project

A. Immunopathology and Immunochemistry

Research efforts in the DMR have been confronted by a major

constraint which is limiting the scope of the studies. This is the knowledge, expertise and the facilities for advanced technology especially in the field of Immunopathology. Further development will enalbe DMR to conduct important research on etiology and pathogenesis.

Development in these areas can be utilised with great advantage in the research on other tropical diseases as well. Thus sound expertise and infrastructure for the fruitful basic research will have to be established, and on the basis of which fresh research efforts may be further launched.

In the field of Immunopathology further development should include the following:

1. Establishment of immunocytochemical and histochemical techniques for identification of RNA, DNA or altered cellular components in liver biopsies.
2. Establishment of immune-electronmicroscopy (IEM) for identification of viral antigens, antibodies, or complexes in liver biopsies.
3. Establishment of immunoperoxidase and immunofluorescent techniques.
4. Establishment of scanning electron microscopic techniques for the morphological characterization of viral particles or agents causing Non-A, Non-B hepatitis.
5. Establishment of techniques for detection of minute quantities of viral antigens, antibodies and immune complexes in the sera.
6. Establishment of an animal model of Non-A, Non-B hepatitis.
7. Further development in the interpretation of ultrastructural and histological features of Non-A, Non-B hepatitis.

8. Development and establishment of the methods used for detecting circulating immune complexes.

Establishment of these techniques cannot take place in a vacuum and therefore these techniques will be used as part of the research activities on Non-A, Non-B hepatitis, liver cancer and rotavirus infections. In order that expertise in these techniques can be obtained a deliberate attempt will be made to utilize them even though it may be a necessary component of the research plan for these diseases.

B. Recombinant DNA technology

Recombinant DNA technology finds wide applications in tropical diseases and biomedical research. Introduction of micro techniques and availability of micro-organisms which can be handled under relaxed safety guidelines made the biotechnology within the reach of scientists in developing countries.

Department of Medical Research, Burma, has adequate infra-structure and research areas for absorbing advanced biomedical technologies including genetic engineering.

The followings are applications of which recombinant DNA techniques will be used within this project.

1. Cloning AFP gene for bulk preparation of anti-AFP globulin.

Production of AFP by recombinant technology appears feasible, and economical in the long run. It is an appropriate candidate for exercise in establishing DNA cloning techniques in DMR.

- see also 6(A)

2. Construction of radio-labelled gene probes for detecting LT, ST producing E. coli

Radiolabelled gene probes for screening LT and ST producing E. coli have been used in DMR with success. The probes were provided by collaborating laboratories and it is felt that construction of the probes from the privided gene "chips" by Nick translation for future and extended use in DMR is necessary and feasible.

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C. Infrastructure support

To facilitate effective and competent viomedical research it would be important to build-up and provide the infrastructure support simultaneously during various stages of the collaboration.

Infrastructure developments and promotion would be directed towards further strengthening of the (1) laboratory animals services (2) repair and maintainance services and supportive services.

10. RESEARCH ON BACTERIOLOGY OF ACUTE DIARRHOEA

Research Objective/activities

A. Biochemistry of Toxins produced by Enteric Pathogens

(i) Biochemical characterization of Shigells Toxins:

The elucidation of the cellular mechanisms of action of the becterial protein toxins remains a complex problem. Cytotoxic, neutrotoxic and enterotoxic activities of Shigella toxin have all been demonstrated and enterotoxic activities of Shigella toxin have all been demonstrated in Shigella culture media and bacterial lysates. A number of workers have studies the effects of partially purified shiga toxin in Hela cell system for cytotoxic property. In contrast, a number of cell lines are highly resistant to shiga toxin. One possible mechanism for such resistance is a lack of cell surface receptors for the toxin. A number of lines of evidence suggest that shiga toxin enters sensitive cells by a process of RME (Receptor mediated endocytic) uptake. It has been shown that a receptor-binding step is a prerequisite for the expression of biological activity and the extreme toxicity of shiga toxin.

The halotoxin of shigella has a MW of approximately 72,000 daltons and contains a 30,000 daltons subunit. The presence of smaller 4 - 7,000 or 11,000 subunits have also been reported. But the precise size, structure and composition of toxin are not yet certain, apart from the structure of native cytotoxin which has only recently been elucidated.

We believe that toxin plays a crucial role in pathogenesis of both watery diarrhoea and colonie dysenteric phases of the disease. The mechanism by which it causes intestinal secretion or cell death is under active investigation.

LT or ST. Although very little direct information is available on the internalization of LT by target cells, presumably the mecahnism is essentially the same as that of cholera toxin. Furthermore, no evidence was obtained for toxin-catalysed ADP-ribosylation of cytoskeletal proteins in intact cells, although cytoskeletal proteins appear to be targets for ADP ribosylation by cholera toxin.

Moreover, though both cholera toxin and E. coli LT bind to GM_1 from the experiments it appears that there are distinct differenced in their binding specificities. Most notable is the much higher binding affinity of E. coli LT than cholera toxin for galactose-containing supports. This observation suggest that the receptors for these two toxins may be different and is consistent with the proposal that a galactoprotein may be the E. coli LT receptor (Halmgren & Lonnroth, 1975). It will thus be of interest to determine the structure of the receptor-finding domain(s) on the B chanis of these two toxins.

E. coli ST toxin is quite small (approx. 2000 MW) and apparently elicits its enterotoxic response by stimulating guanylate cyclase. Whether or not heat stable toxins enters cells to carry out its action is not clear. Furthermore, no enzymatic activity has been reported for heat stable toxin. Considering the toxin size, it is much more likely to act stoichiometrically that catalytically, but this remains to be determined.

Work Plan

1. Distribution and binding efficiency of LT in CHO cell assay
2. Phosphorylation of protein by LT toxin in CHO cells
3. Purification and characterization of ST and further elucidate its role in isolated cells.

Work Plan

We intend to isolate and purify the subunits of shigella toxin and find out their biological effect and pathogenicity.

(ii) Development of method for identification of toxin produced by diarrhoegenic shigella

The shigella organisms induce a severe diarrheic and dysenteric syndrome (shigellosis) which is mediated by a protein exotoxin. A role for toxin or toxins in pathogenesis of both major shigella intestinal syndromes, diarrhoea and dysentery is very likely. In-vivo microcolony growth and localized toxin production within invaded cells of the colonic epithelium may lead to extensive cellular injury and expressed clinically as colitis and dysentery.

Shiga toxin may also produce secretion of water and electrolyte which could account for the characteristic diarrhoeal phase. At the present time, it is not clear what role the toxin plays in the pathogenesis of the disease, especially in the diarrhoeal phase. No one can say exactly, to what extent this toxin plays in the diarrhoea phase. So it is necessary to detect the toxin produced by shigella bacillus especially in upper part of the gut.

Work Plan

We intend to isolate shigalla bacillus colonizing in upper gut in cases of diarrhoea and to find out whether these isolates secrete toxin and whether these isolates cause secretory response in various test systems.

(iii) Further work on the biochemical properties of LT and ST:

Objective and Rational

There is now much evidence that most cases of acute diarrhoea are caused by infection with certain toxigenic strains of E. coli,

B. Serotyping of Escherichia coli:

Research Objective/activities

i. Serotyping 'O' and 'H' of isolates from Burma to find out serotype prevalent in E. coli from Burma.

ii. To serotype untypable serotypes form Burma

iii. To develop, raise and store antisera against known serotypes in order to become a reference centre.

Objectives and Rational

Enterotoxigenic E. coli strains tend to belong to a limited number of serotypes. Orskov and Orskov, 1980 also studied 388 ETEC strains from many geographical areas and reported that 0 groups (06, 08, 015, 020, 025, 078, 0115 and 0159) were found most frequently and accounted for 242 (62 per cent) of the isolates. Merson et al., 1980 using antisera for 11 E. coli 0 groups were able to type 64 per cent of ETEC examined in Bangladesh. Rowe et al., 1983 carried out the serotyping of ETEC from various Asian countries and reported that 60 per cent belonged to the same 11 serogroups.

One consequence of the clear-cut demonstration of the pathogenicity of enteropathogenic E. coli strains and recognition of viruproperties is a rekindling of interest to study the epidemiology of enteropathogenic E. coli infection.

Work Plan

1. To send to a Reference Centre in Japan - isolate for conslate serotyping of E. coli isolation

or

2. To obtain serotyping antisera for the Reference Centre in Japan for constalation serotyping of all isolation of E. coli.

11. ANNEX

Department of Medical Research, Ministry of Health

PROJECT PROPOSAL

1. Name of Project	Research on the Treatment of Infectious Diseases of the Alimentary System.
2. Name of implementing agency (Government)	Department of Medical Research, Ministry of Health
3. Name of co-operation agency	Japan International Co-operation Agency (JICA)
4. Project site	Clinical Research Center and Bio-Medical Research Center of the Department of Medical Research, No. 5, Zafar Shah Road, Rangoon, Burma.
5. Sector and development	The Government of Japan and the Government of the Socialist Republic of the Union of Burma will co-operate with each other in implementing the project. The project aims to contribute advanced knowledge and insight into the biological processes underlying. Infectious Diseases of the Alimentary System and improve specific therapeutic and control measures and thus promote health conditions in the Socialist Republic of the Union of Burma.
6. Project objectives	The project aims to conduct research on viral and parasitic infections of the liver and gut and their sequelae such

	as cirrhosis and primary liver cancer. The goal of the comprehensive studies will be to achieve improvement and diagnosis, prevention and treatment of diseases due to these infections in Burma.
7. Activities under the project	1. Research in the characterization of some of the newer Hepatitis viruses and their pathogenicity for effective implementation of therapeutic and control measures. 2. Research in defining and characterization of known and potential enteric viral pathogens causing diarrhoea and the mode of transmission and pathogenicity of rotavirus. 3. Research in the parasitic infections of the liver and gut. 4. Further development of advanced technology and support services for relevant research activities.
8. Project study status	Preliminary survey to be initiated in 1984 in order to commence Project in April 1985.
9. JICA contribution	Tentatively estimated as 50-100 million Yen for the first 2 years and 50-100 million Yen in the next 2 years.
10. Burma Government contribution	Part of existing laboratory facilities and staff.
11. Implementation period	1985-1987 for years and to be extended up to 4 years till 1989 if necessary according to the research development.

12. Justification

a) In Burma mortality due to diarrhoea ranks first in the list of diseases and it is No.2 in the morbidity. Preliminary studies have revealed that Rotavirus, and Enterovirus, accounts for about 20-30 percent of acute diarrhoea in childhood.

b) Parasitic agents like Entamoeba histolytica and helminths also commonly cause diseases in the liver and alimentary system and are responsible for malnutrition in children and significant morbidity and mortality in all age groups.

c) Viral Hepatitis is a major health problem in Burma. Is is an endemic disease with occasional outbreaks of epidemic. Studies have revealed that apart from hepatitis A and hepatitis B virus, evidence of the presence of hepatitis Non-A, Non-B virus have been found since 1982. Viral Hepatitis B is a preventable cause of chronic liver diseases and primary hepatocarcinoma. Hepatitis virus Non-A, Non-B causes high fatality among pregnant women.

The major public health importance of the infectious diseases of the Alimantary

System in Burma as examplified by viral diarrhoeas, amoebic dysentry, amoebic hepatitis, viral hepatitis and intestinal helminthiasis,

- the strong possibility that more intense research in Burma will improve their diagnosis, treatment and control and the existence at the Department of Medical Research of basic laboratory facilities (Biomedical Research Center-donated by the Japanese Government) and scientific manpower.
- indicates that the proposed research co-operation will be worthwhile and cost effective.

資料－2.

A CLINICAL AND EPIDEMIOLOGICAL STUDY OF AN EPIDEMIC OF NON-A, NON-B HEPATITIS IN RANGOON

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Abstract

Of a total of 519 cases of viral hepatitis admitted to the Infectious Diseases Hospital from June to October 1982, during an epidemic in Rangoon, 399 cases were found to be hepatitis non-A, non-B, 84 cases were hepatitis B and 36 cases were hepatitis A. A clinical study on 399 non-A, non-B hepatitis cases was done. Also a prospective study of 434 households made up of 217 non-A, non-B hepatitis cases with their families, together with 217 matched control families were followed up for a period of seven months to detect secondary cases among the family members. Non-A, non-B hepatitis was found to occur most in adults of second, third and fourth decades. Non-A, non-B hepatitis is indistinguishable from other two types of viral hepatitis. Case fatality rate was the highest in pregnant women with non-A, non-B hepatitis. The field study suggested non-A, non-B hepatitis can be transmitted by intrafamily spread. No evidence of sexual or syring transmission of non-A, non-B hepatitis was found.

MATERIALS AND METHODS

Subjects

399 patients who had clinical features of hepatitis and elevated liver function tests, who were also negative for serological markers of hepatitis A and B, and without evidence of leptospira and malaria infections were diagnosed as having non-A, non-B hepatitis and served as subjects for the clinical study.

217 non-A, non-B hepatitis index cases who were accessible and living in Greater Rangoon Area, and for whom matched controls could be found in their neighbourhood, were included in the field study.

The screening of cases in the study is as follows. A total of 527 patients admitted to the Infectious Diseases Hospital between June and October 1982, presenting with jaundice of less than one week duration and with clinical features of acute hepatitis were screened. 8 cases were found to be leptospirosis and were excluded. From the remaining 519 cases diagnosed provisionally as viral hepatitis, 84 cases (16.2%) had serologic markers for hepatitis B, and 36 cases (6.9%) had hepatitis A. The remaining 399 cases (76.9%) were then diagnosed as non-A, non-B hepatitis by exclusion, and registered for the clinical study. All those cases diagnosed as non-A, non-B hepatitis who were accessible and living within Greater Rangoon Area were traced to their residential addresses, and their households registered in the field study as index households. These households were follwed

INTRODUCTION

Viral hepatitis is highly prevalent in Burma. Epidemics occurred in Mandalay[1] in 1976-77 and in Moulmein[2] in 1978.

During the early part of 1982, the number of hepatitis cases attending the out-patient department of Urban Health Centers and those admitted to the Infectious Diseases Hospital (IDH), Rangoon, rose very steeply. Preliminary study of cases admitted to the hospital revealed a majority to be of the non-A, non-B type. The occurrence of this epidemic has provided an opportunity to recognise and study cases of non-A, non-B hepatitis for the first time in Burma.

This study was undertaken with the objectives of describing the clinical and epidemiological features of non-A, non-B hepatitis especially to determine (a) whether the clinical features differ from other forms of viral hepatitis, (b) whether non-A, non-B hepatitis transmitted by intrafamily spread, and (c) whether parenteral and syringe transmission has a role in the present outbreak of non-A, non-B hepatitis in Rangoon.

up weekly together with their counterpart control households living in the same neighbourhood, who were matched individually for age group, sex, marital status and household-wise for family size and proportion of children in the household, to detect secondary cases emerging from the study population. Excluding cases that had expired, those cases having other family members suffering from jaundice within previous six months, and those cases for whom suitably matched control households could not be found in the same neighbourhood (eg. single persons, monks, persons living in monasteries and pregnant women), a total of 217 pairs of index and control households were successfully followed by weekly household visits for a period of seven months.

Laboratory Diagnostic Methods

As there is no serological tests specific for the diagnosis of non-A, non-B hepatitis, diagnosis is mode on the grounds of exclusion of other known causes of acute hepatitis. In all cases admitted with jaundice and clinically diagnosed as viral hepatitis, investigations for liver functions, tests for hepatitis B surface antigen(HBsAg), antibody to hepatitis B core antigen(anti-HBc), antibody to HBsAg (anti-HBs), and IgM antibody to hepatitis A virus (anti-HAV-IgM) were performed. Tests for liver functions include serum bilirubin, serum alanine aminotransferase (ALT), and serum aspartate aminotransferase (AST). HBsAg, anti-HBc, anti-HBs and anti-HAV IgM were determined by radioimmunoassay (RIA, ABBOTT) in the National Hepatitis Laboratory at the Department of Medical Research. Serological tests for leptospira were done at the

National Health Laboratory, and blood films were examined for malaria parasites.

RESULTS

Clinical Features

Of the 519 studied patients, 36(6.9%) had hepatitis A, 84 (16.2%) had hepatitis B, and 399 (76.9%) had hepatitis non-A, non-B (Table 1). Mean age of hepatitis non-A, non-B was 28.6±0.6 (SEM) years, and that for hepatitis A and B were 10.2±1.3 years and 26.8±1.5 years respectively. More males were affected by all types of hepatitis, particularly by hepatitis A. Mean serum bilirubin for hepatitis non-A, non-B was 149.7±8.9 umol/L and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were 157.7±5.3 IU/L, and 149.2±4.6 IU/L respectively. These biochemical findings were similar to those found in other two types of hepatitis.

Clinical features such as jaundice (100%), anorexia (72.2%), abdominal pain (67.9%), nausea (59.4%), vomitting (49.6%), itchiness (12.5%), diarrhoea (3.5%), dark coloured urine (100%), hepatomegaly (100%), and splenomegaly (0.5%) were observed in hepatitis non-A, non-B. These features were also common in other types of viral hepatitis. Urticarial rash and arthralgia were not seen in any patient with viral hepatitis. Fulminant hepatits occurred equally in hepatitis non-A, non-B (15/399 = 3.8%) and hepatitis B (4/84 = 4.8%), but there was not a single fulminant case of hepatitis A. Serum bilirubin, ALT and AST levels in

fulminant hepatitis non-A, non-B were 245.8±56.6 uml/L, 161.3±27.1 IU/L and 168.9±23.6 IU/L respectively, and were much higher than those of non-fulminant cases. Similar results were found with hepatitis B. With the exception of one non-pregnant female with non-A, non-B hepatitis, all other fulminant hepatitis patients died of hepatic failure.

Case fatality rate among hepatitis B in our study ranged from 4.2% to 6.3% and was similar in males, non-pregnant females, and pregnant females (Table 2). It was about four times higher than non-A, non-B hepatitis in males and non-pregnant females; but only half that of non-A, non-B hepatitis in pregnant females.

One male patient from the non-A, non-B group was readmitted three months after discharge from the hospital. He had typical prodromal symptoms of another attack of viral hepatitis and markers for acute hepatitis A and B were absent.

Results of the Field Study

During the seven month follow up of 2779 individuals from the 217 pairs of index and control households, a total of 15 jaundice cases were identified. 11 cases were from the index households, of which one case was hepatitis B, and the remaining 10 were non-A, non-B hepatitis. A total of 4 jaundice cases emerged from the control households, of which 2 were hepatitis B, and the remaining 2 were hepatitis non-A, non-B. There was no case of hepatitis A or other causes of jaundice diagnosed among the secondary cases. Thus, the incidence rate or secondary attack rate of non-A, non-B hepatitis in the exposed

index household members is calculated to be 7.7 per 1000 population, and the incidence in the non-exposed control household members is 1.3 per 1000 population (Table 3). This difference is statistically significant at a P level of $.01 < P < .02$. The relative risk of acquiring non-A, non-B hepatitis in the presence of an exposure among a household member is six times that of non-exposed control household members.

An attempt was made to analyse the relationship of secondary cause to their respective index cases ; 6 cases (60%) occurred among siblings, 1 case was a nephew, and 3 cases bore no blood relationship to their index cases. There were no secondary cases among the marital partners in this study.

A retrospective enquiry into past history of injection or any form of needling was made. A comparison of rate of exposure to injection between non-A, non-B hepatitis cases (217 index cause plus 12 secondary cases) and controls revealed that the rate of exposure to injection was almost equal in the two groups, with a rate of 16.5% in the case group and 16.6% in the controls (Table 4).

DISCUSSION

With the availability of specific serologic markers for hepatitis A and B, this recent outbreak of hepatitis epidemic in Rangoon has provided an opportunity to recognise and study non-A, non-B hepatitis for the first time in Burma.

The age pattern of patients affected in this epidemic, as reflected by cases admitted to the Infectious Diseases Hospital during the study period (June to October 1982), is seen to be distinctly different from the pattern found in yearly admitted cases of viral hepatitis to IDH previously in 1974 and 1975 (Table 5). The distinctive feature in previous years was the occurrence of a high proportion of cases in the 0-9 years age group, probably due to predominance of hepatitis A affecting the young children, whereas in the present epidemic, the largest proportion of cases occurred in young adults of second, third and fourth decades. This finding is consistant with Khuroo's study of a Kashmir epidemic[3], Wong's study of a Delhi epidemic[4], and Kane's report on a Nepal epidemic[5].

Our finding of an almost equal sex ratio in non-A, non-B hepatitis is not in conformity with the reports of Kane et al[5]., who found a M : F ratio of 3 : 1 and Khuroo et al[3]., who found a M : F ratio of 1 : 0.8 in Kashmir epidemic. However, our finding of an equal ratio could be due to an over representation of the female population by pregnant women. It was known from previous experience of a Mandalay epidemic that there is increased severity and fatality among pregnant women of which both doctors

and the population are acutely aware. This may have prompted early precentation of hepatitis cases among pregnant women, and doctors may be admitting less serious pregnant cases of hepatitis who would otherwise have had outpatient care only.

The hepatitis epidemic which occurred in Mandalay[1] in 1976-1977 was presumed to be due to hepatitis A because of one absence of serologic markers for hepatitis B in even the most severely ill patients during the epidemic, the absence of any history of parenteral exposure in affected persons, and because the epidemic occurred after flooding of Shwe-ta-chaung canal which caused contamination of water supplies in the area. Later, the epidemic was speculated to be due to a different virus[6]. That epidemic can now be retrospectively concluded as due to non-A, non-B hepatitis for the following reasons : (1) the age distribution of affected person shifting to older population than the usual 0-9 years age group (2) absence of serologic markers of HBV in a sample of cases, and (3) antibodies to hepatitis A are now known to be acquired in very early ages in Burma, 98% of children by 8 years of age have acquired immunity to HAV[7], and thus the age groups affected in the epidemic were those that would have already acquired immunity to hepatitis A.

For similar reasons, the Moulmein epidemic of hepatitis[2] in 1978 can retrospectively be speculated as having been due to non-A, non-B hepatitis.

At present, insufficient information exists to classify the non-A, non-B hepatitis agents. It has been speculated that

there exist at least two agents ; one mimicking HBV mainly parenterally transmitted and the other mimicking HAV transmitted by the faecal-oral route and responsible for epidemics. Each of the two categories could include more that one agent[8]. Epidemic non-A, non-B hepatitis is though to be spread by ingestion of contaminated substances, especially water[3,4, 9-12]. Faecal-oral spread of non-A, non-B hepatitis has been demonstrated by Balayan et al[13], by experimentally administering concentrated faecal material from the acute patients with faecal-oral non-A, non-B hepatitis, to a human volunteer who developed non-A, non-B hepatitis after an incubation period of 36 days.

The presenting clinical features of acute non-A, non-B hepatitis in this study were indistinguishable from those of hepatitis A or hepatitis B, and were similar to those reported by Khuroo, et al[3]., in the study of an epidemic of non-A, non-B hepatitis in Kashmir , India.

Rash and arthralgia were absent in all our patients, including the hepaitis B cases. However, Khin Maung Win[14] had described the presence of arthralgia in viral hepatitis patients in the epidemic of Mandalay in 1976-1977. Similarly, Lucas, et al[15]., and Perillo, et al[16]., reported that rash and arthralgia were present in some non-A, non-B hepatitis patients.

Biochemical findings were similar in all three types of viral hepatitis in our study. Bamber, et al[17]., in the study of a hospital population in London, showed that peak serum bilirubin and AST levels in non-A, non-B hepatitis was lower than that of hepatitis A and B. However, in their study of

acute sporadic non-A, non-B hepatitis in India, Khuroo, et al[18]., had described the serum bilirubin level to be the same as hepatitis B, but higher than hepatitis A. Although mean serum bilirubin, ALT and AST levels in our patients with fulminant hepatitis were much higher than uncomplicated cases, they were equally high in all types of viral hepatitis In their study of fulminant hepatitis in a London hospital, Gimson, et el[19]., found that maximum levels of serum bilirubin and AST in non-A, non-B hepatitis were much higher than in hepatitis A and B. The reason for the difference between our data and theirs may be due to the fact that ours was based upon the results of a single testing at admission and were not maximum values.

Khuroo, et al[11]., found that fulminant hepatitis was significantly high among women in the third trimester of pregnancy, compared to non-pregnant and male groups. Similary, poor maternal outcome was reported by Naidu, et al[20]., in Delhi epidemic and Borhanmanesh, et.al[21]., from Iran, and Kane et al[5]., reported a 21% mortality rate in pregnant women in their study of non-A, non-B hepatitis in Nepal.

In our series, the highest case fatality rate was observed in pregnant women with non-A, non-B hepatitis as compared to hepatitis in males or non-pregnant females, or when compared to hepatitis caused by A or B virus. This feature of conspicuously high mortality among pregnant females with non-A, non-B hepatitis, in contrast to non-pregnant females or males with hepatitis A or B infections is noteworthy. It is not known whether non-A, non-B virus is move virulent in pregnant women, or whether immune responses are

impaired in non-A, non-B infection during pregnancy.

In the epidemic of viral hepatitis in Mandalay[1, 22, 23], it was reported that the case fatality rate in pregnent women was six fold higher than the overall case fatality rate (CFR) and about twice that for non-pregnant women. It was shown that pregnancy in the third trimester was the period when most fatalities occurred. Their findings match the results of the present study. As it is virtually certain now that the Mandalay epidemic was due to non-A, non-B virus, the observations made with regard to high fatality among pregnant women may be considered as due to non-A, non-B hepatitis.

The finding of a significantly higher secondary attack rate within household members of index cases, compared to comparable control households without hepatitis cases (7.7 per 1000 and 1.3 per 1000 respectively) suggest person to person spread within household contacts by the faecal-oral route. Although previously documented epidemics of non-A, non-B hepatitis were common source water-borne epidemics such as those of Delhi, Kashmir and possibly also in Mandalay, no single water source could be incriminated in this study, even though faecal contamination of water supplies due to impaired drainage and leakages of pipe lines are always possible.

No evidence of sexual transmission could be documented from our study as there were no secondary cases among the marital partners of index cases. Khuroo, et al[18]., and Bamber[17], also did not find evidence of sexual transmission in theri study of non-A, non-B hepatitis.

Parenteral and syringe transmission definitely did not play a role in the transmission of epidemic non-A, non-B hepatitis outbreak in Rangoon. These are also consistant with the findings of Delhi epidemic, Kashmir epidemic and Pune epidemic, all of which were epidemic non-A, non-B hepatitis outbreaks[24].

Reviewing the age pattern of viral hepatitis cases admitted to IDH from 1974 to 1982 (Table 5), it was found that the change in age pattern was not dramatic but gradual, starting in 1976. It is tempting to speculate that non-A, non-B hepatitis was introduced into the population of Rangoon in 1976 at the time of the Mandalay epidemic, and maintained in a sporadic form, again reaching epidemic status in 1982.

Thus it may be concluded that the present epidemic of hepatitis in Rangoon is mostly due to non-A, non-B hepatitis and that the epidemic which occurred in Mandalay, Moulmein and other parts of Burma were also due to non-A, non-B hepatitis. In addition it is probable that sporadic cases of non-A, non-B hepatitis are also occurring in Rangoon. Other studies just completed at the Department of Medical Research and which will be reported later indicate that non-A, non-B agents are also responsible for a significant proportion of post-transfusion hepatitis. These consideration and the observation that two episodes of non-A, non-B hepatitis occurred within 3 months in one of our patients indicate that sporadic, epidemic (non-parenteral), as well as parenteral forms of non-A, non-B hepatitis are occurring in Burma.

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Table 1

Clinical and biochemical date of 519 acute viral hepatitis patients

	VIRAL HEPATITIS		
	A	B	Non-A, Non-B
Number	36	84	399
Age-years (mean ± SEM)	10.2±1.3	26.8±1.5	28.6±0.6
(Range)	(3-47)	(3-67)	(4-77)
Male/female ratio	2.6 : 1	1.1 : 1	1 : 1
Fulminant cases	-	4	15
Serum bilirubin (μmol/L) (mean ± SEM)	134.1±13.8	139.3±15.9	149.7±8.9
Serum ALT (IU/L) (mean ± SEM)	150.7±13.3	126.9±10.4	157.7±5.3
Serum AST (IU/L) (mean ± SEM)	144.7±12.5	132.3±7.4	149.2±4.6

Table 2

Case fatality rate (CFR) in non-A, non-B hepatitis

Type of Hepatitis	Male			Female						Total		
				Pregnant			Non-pregnant					
	Case	Death	CFR	Case	Death	CFR	Case	Death	CFR	Case	Death	CFR
A	26	-	-	1	-	-	9	-	-	36	-	-
B	44	2	4.6	16	1	6.3	24	1	4.2	84	4	4.8
Non-A, Non-B	208	2	0.9	84	10	12	107	2	1.9	399	14	3.5

Table 3

Incidence of non-A, non-B hepatitis cases in the index and control household members

Study population	No. of Population	No. of non-A, non-B hepatitis cases	Incidence per 1,000 population	Significance testing
Index households (Exposed group)	1294	10	7.7	
Control households (Non-exposed group)	1485	2	1.3	$0.01 < p < 0.02$
Total	2779	12	4.3	

Table 4

Comparison of rate of exposure to injection in non-A, non-B hepatitis cases and their controls

Study Population	History of Injection			Total
	present	absent	not mentioned	
Non-A, Non-B hepatitis cases	38	191	-	229
(Index + secondary)	(16.59)	(83.41)		(100)
Controls	36	177	4	217
	(16.6)	(81.57)	(1.84)	(100)
Total	74	368	4	446
	(16.59)	(82.51)	(0.89)	(100)

Table 5

Percentage distribution of in-patient viral hepatitis cases admitted to IDH by age groups from 1974 to 1982: compared to that of epidemic of viral hepatitis Mandalay 1976 and Moulmein 1978

Age	Cases admitted to Infecious Diseases Hospital, Rangoon									Mandalay	Moulmei
	Endemic Years		Transition Period						Epidemic	Epidemic	Epidemi
Groups	1974	1975	1976	1977	1978	1979	1980	1981	1982	1976	1978
0-9 yrs	78	84	40	38	18	47.2	26.3	47.8	7.71	5.9	10.4
10-19	12	10	27	16	17.8	15.8	17.8	16.7	17.15	22.2	13.0
20-29	5	2.6	16	23	28.5	18.4	23.7	18.4	42.0	43.4	17.2
30-39	2.7	2.0	8	11	16.5	7.7	14.5	6.0	16.76	15.7	19.8
40-49	1.7	0.6	4	7	11.3	5.9	10.3	5.6	9.44	8.4	13.5
50-59	0.3	0.4	3	3	5.5	3.1	4.9	3.6	4.62	2.6	13.0
60 +	0.3	0.4	2	1.3	2.4	1.9	2.5	1.9	2.31	0.7	13.0

Footnotes :

For Table 1 μmol/L = micro mole per litze

For Table 4 The number in parentheses represent the percentage in row.

For Table 5

Source : 1974, 1975, 1976, 1977 figures extracted from paper presented at Inter-Regional Seminar on Viral Hepatitis, Kuala-Lumpur ; November 1977 by Dr. Khin Maung Tin.

: 1978, 1979, 1980, 1981 figures calculated from available registers of in-patients at IDH.

: 1982 figures represent those cases admitted to IDH during the study period.

: 1976 Mandalay epidemic figures extracted from Hepatitis Scientific Memoranda. H-1336/1, p.7 : Dec., 1977 by Khin Maung Tin and Myint Myint Khin.

: 1978 Moulmein epidemic figures represent community survey figures in one ward of Moulmein during viral hepatitis epidemic, Sein Oo et al., 1980.

Note : The age interval used to classify viral hepatitis cases in 1974, 1975, 1976, 1977 and 1976 Mandalay epidemic was 10 years intervals. eg. 0-10, 11-20, 21-30 etc. etc.

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