ビルマ消化器感染症研究プロジェクト事前調査団

報　　告　　書

昭和60年 4 月

国 際 協 力 事 業 団

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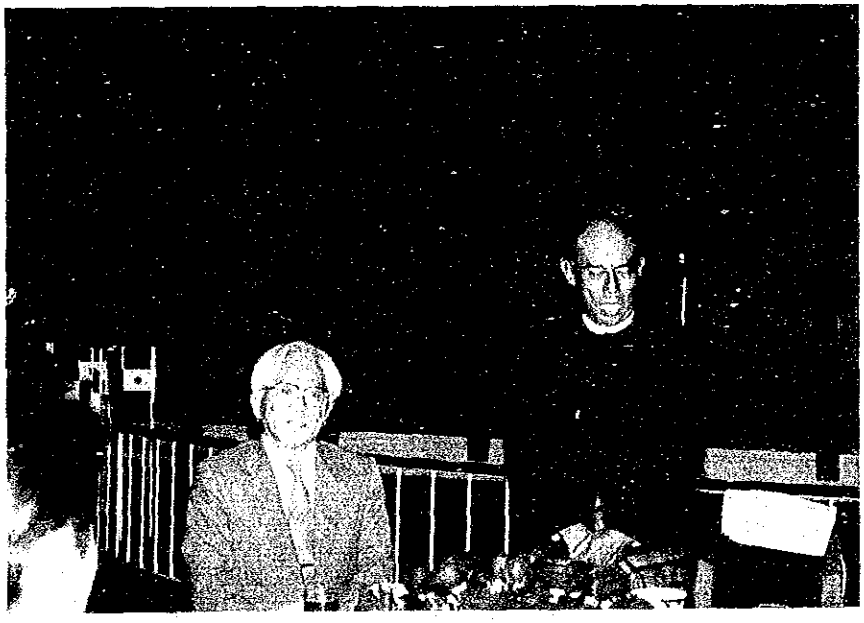
塚本政雄大使表敬訪問　　於　日本大使館　（昭和６０年２月１９日）
左より今井団員、中根教授、江見専門家、濱島教授、塚本大使、畑中教授、村田ＪＩＣＡ担当官、新田参事官



挨拶するＵ Tun Wai 保健大臣　（２月２５日　インヤレークホテルにて）

事前調査打合せ会議

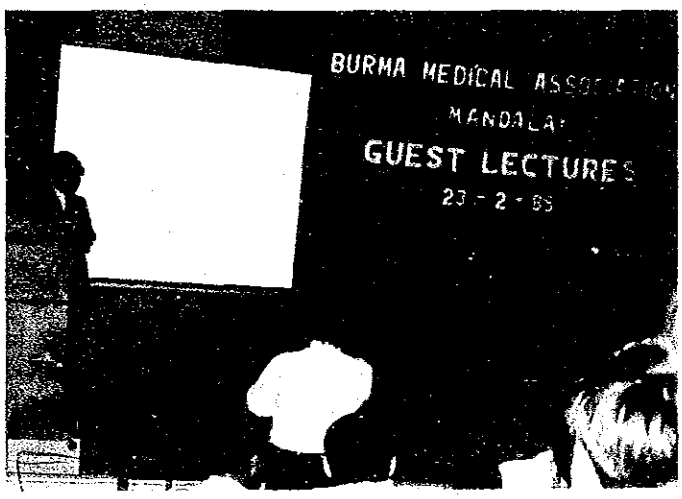
ビルマ研究局スタッフと。生物医学研究センター会議室にて。

WHO/DMR Reserch Seminar on Recent Studies of Diarrhoeal Diseasesに出席した団員　（昭和６０年２月２２日、生物医学研究センター図書館カンファレンスルームにおいて）

WHO/DMR Seminarで研究発表に質問する濱島団長

マンダレー総合病院臨床講堂での３教授による招待講演（昭和６０年２月２３日）

講演后、質問を受ける畑中教授　（質問するはDr．Saw Mya Yi）



中根教授の講演。卓越した英語で聴衆を魅了。（質問しているのはDr．Hla Oo）

講演中の濱島教授

ま　え　が　き

ビルマ国に対するわが国の医療協力の一環として，同国の医学研究所のウィルス部門の整備拡充を図るため，昭和４２年度より５年間にわたりプロジェクトタイプの技術協力が実施された結果，各種のウイルス性疾患の調査，研究の進展に多大の成果があった。

その後，わが国の無償資金協力により昭和５５年３月に完成した生物医学研究センターの施設を活用し，同国の感染症に対する生物医学的研究の発展に寄与することを目的として，感染症研究対策プロジェクトが昭和５５年４月１０日より４年間にわたり実施され，ウィルス学部門，細菌学部門及び免疫学部門において目ざましい成果を収めた。

ビルマ政府はこれまでのわが国の医療協力を高く評価し，改めて肝臓および消化管のウイルス性ならびに寄生虫性疾患に対する研究につき協力を要請してきた。

以上の経緯を踏まえて，当事業団は要請の具体的内容を調査し，本件協力の可能性を検討するため，昭和６０年2月１７日より３月１日まで，京都大学医学部濱島義博教授を団長とする事前調査団を派遣した。本報告書はその調査結果をまとめたものであり，ここに本調査団団員各位並びに同調査団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ります。

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ります。

昭和６０年４月

国　際　協　力　事　業　団

理事　　末　永　昌　介

目　　次

I. 事前調査団派遣の経緯

ビルマ国政府は，ビルマ国立医学研究局における新しいプロジェクト「消化器系の感染症」の技術協力を正式にわが国政府に要請して来た。わが国としては，このビルマ国医学研究局に対して，去る昭和４３年（１９６８年）より昨年に到るまで長期にわたり技術協力をして来たところのものであり，かつ昭和５４年１１月には日本政府無償供与による「生物医学研究センター」の開設をみ，これを使用しての「感染症プロジェクト」の技術協力が昭和５５年（１９８０）４月１０日より昭和５９年（１９８４）４月９日まで行われて来たものである。前回のプロジェクトの終了に先立って去る昭和５８年（１９８３）１２月１８日より同１２月２７日まで，このプロジェクトに対する日本側のビルマ側への技術協力の成果を評価するべく田中健蔵九大学長を団長とし，評価調査が行われた。

そのエバリュエーション調査報告では，さらに同センターに対するわが国技術協力は継続する必要があり，より強力な援助を必要とするものと報告している。

昭和５９年１１月１日，ビルマ国政府は本医学研究局における技術協力の要請を新しいプロジェクトとして「Research on the Treatment of Infectious Diseases of the Alimentary System」として正式に申し出て来た。

これに対して，この新しい要請に関する調査のために濱島義博京大教授を団長とする事前調査団を昭和６０年（１９８５）２月１７日より同３月１日まで派遣した。

ビルマ国からの要請時のビルマ側の Project Outline は次の如きものである。

Department of Medical Research
Ministry of Health

PROJECT OUTLINE

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 cooperating agency	Japan International Cooperation 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 objectives	The Government of Japan and the Government of the Socialist Republic of the Union of Burma will cooperate 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/ description

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 is to achieve improvements in diagnosis, prevention and treatment of diseases due to these infections in Burma.

A consideration of these objective will show that the disease problems chosen are of public health importance to the country.

Also, the particular situation in Burma of some of these diseases offers unique opportunities and challanges which may lead to findings of scientific significance and practical benefit.

These disease problems include epidemic non-A, non-B hepatitis, cirrhosis and primary liver cancer, hepatic amoebiasis, and rotavirus infections of the gut.

The multidisciplinary approach necessary will mean that basic disciplines such as immunopathology will be developed to a stage where they will provide a sound foundation for future research efforts.

7. Activities under the project

1. Research in the characterization of some of the never Hepatitis viruses and their pathogenicity for effective implementation of therapeutic and control measures.

a) Epidemic non-A, non-B hepatitis:

Viral hepatitis is highly prevalent in Burma. Among various types of hepatitis, recently discovered epidemic non-A, non-B hepatitis has occured in the form of major epidemics in Burma e.g. the largest epidemic in Mandalay in 1976-77 involving 20,000 icteric cases, the epidemic in Moulmein in 1978 and the recent epidemic in Rangoon in 1982 with an approximately 8,000 reported cases. Unlike the parenterally transmitted non-A, non-B hepatitis which is widespread through out the world the recent outbreaks of epidemic non-A, non-B are still confined to our area. The mode of transmission in these epidemics has been attributed water and food, and also person to person spread.

Case fatality rate is the highest 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.

The attempt to identify and isolate the definite aetiological agents have not been successful yet. A preliminary immune electron microscopic study of stools from Burmese patients has shown virus-like particles of 27 mm size. This promising findings should be thoroughly followed up by further investigations.

b) Hepatitis B virus and Chronic Liver Diseases:

Viral hepatitis B is a major health problem in Burma where 10-15 per cent of general population are HBsAg carriers and 45 per cent have anti-HBs antibody. HBsAg positive carrier mothers are the major source of infection as 60-70 per cent of the infants born to HBsAg/HBeAg positive mothers become HBsAg carriers within one year.

c) Chronic liver diseases such as cirrhosis and primary liver cancer are prevalent in Burma. It is 5th in ranking among the single leading causes of mortality at the Rangoon General Hospital. Primary liver cancer is the second commonest cancer in males. Retrospective studies on chronic liver diseases in adults as well as in children, by performing autopsies and using Shikata's Stain, have shown that a substantial number of chronic liver diseases were associated with hepatitis B virus in the liver tissue. The HBV has been attributed as the causal agent in 80 per cent of primary liver cancer patients. The clinico-epidemiological studies on cirrhosis and primary liver cancer had been conducted to some extent by the Department of Medical Research. However, there are many unexplored areas of research such as pathogenic mechanisms, clinical trials of newly discovered

chemotherapeutic agents or a surgical approach, etc. The fact that the Department of Medical Research has facilities for determining hepatitis virus markers, electron microscopic studies, animal experiments and patients care responsibilities have created a good research environment where an appropriate input for the matter can result in beneficial scientific findings of major importance.

2. Research in defining and characterization of known and potential enteric viral pathogens causing diarrhoea and the mode of transmission and pathogenecity of rotavirus.

It has been well established that rotavirus is the most important single pathogen implicated in diarrhoea among children under three years of age in this country. Rotavirus has been found to be associated with 25.6% of diarrhoea cases in the 1 - 11 month age group and 14.4% in the 12 - 35 month age group. Although the aetiologic role of rotavirus in childhood diarrhoea has been well defined, a number of important questions remain unanswered. The mode of transmission of rotavirus and the pattern of spread within families and communities have not been well delineated. Much remains to be learnt about the immune response to the disease. The number of

clinically important serotypes of rotavirus and their prevalence in different parts of the world are of epidemiological interest. Studies on the prevalence of different rotavirus serotypes and electropherotypes need to be carried out in this country for further classification of the epidemiology of rotavirus infections. This will enable DMR to determine how many virus strians circulate in a family community or country at any given time. It can also be used to follow mode of disease transmission. Rotavirus vaccines are being developed in advanced laboratories and DMR should be able to participate in the early Phase II and Phase III trials of the vaccine. Preliminary studies of the immune status of population with regard to Rotavirus should be done. Although rotavirus has been firmly implicated as an aetiologic agent of diarrhoea, 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.

3. Research in the parasitic infections of the liver and gut.

Dysentery due to Entamoeba histolytica and hepatic amoebiasis are common diseases 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. Recent experimental works had advocated the role of protective immunity in hepatic amoebiasis.

It is of special interest and importance for the country to investigate into this problem with a view to study immuno-pathological aspects and improving diagnostic and treatment measures.

4. Further development of advanced technology and support services for relevant research activities.

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 immuno-pathology. Further development in Immunology and Pathology will enable DMR to conduct

		important research on aetiology and pathogenesis. Development in these areas can be utilized 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.
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 2 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 an Enterovirus accounts for about 20-30 per cent 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. It 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 disease and primary hepatocarcinoma. Hepatitis virus Non-A, non-B causes high fatality among pregnant women.

d) The major public health importance of the infectious diseases of the Alimentary 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 cooperation will be worthwhile and cost-effective.

Ⅱ　調査団の編成と調査日程

Ⅱ－1　調査団の編成

団　長	（総　括）	濱　島　義　博 京都大学医学部教授
団　員	（ウイルス学）	畑　中　正　一 京都大学ウィルス研究所教授
団　員	（細胞生物学）	中　根　一　穂 東海大学医学部教授
団　員	（病院管理）	今　井　辰　雄 京都大学病院事務部長
団　員	（業務調整）	村　田　隆　一 国際協力事業団医療協力部

Ⅱ－2　調 査 日 程

事前調査日程

期　間　昭和６０年２月１７日(日) ～ ３月１日(金)

月　日	曜日	内　　容
2　17	日	13:00　派遣手続及び打合せ（調査団全員），於　竹橋会館 15:15　成田空港着 17:50　TG741（747）成田発 22:30　Bangkok 着
18	月	12:10　Montien Hotel 発 14:50　TG305（AB3）　Bangkok 発 15:30　Rangoon 着 空港では医学研究局のDr. Aung Than Ba Tu 局長，Dr. Khin Maung Tin 所長，Dr. Kywe Thein副所長，Dr. Daw Mi Mi Khin 副所長他多勢のビルマ側，篠浦ＪICA所長などの出迎えを受けて直ちに VIP room へ。 16:40　Inya Lake Hotel チェックイン
19	火	06:45　Meeting （1）

月　日	曜日	内　　容
2　19	火	08:50　大使館へ。 09:10　塚本大使表敬。新田参事官共々打合せ。 10:00　医学研究局着 ビルマ研究者より研究報告を聞き，討論開始。 (Dr. Hla Myint, Dr. Soe Thein, Dr. Myint Lwin, Dr. Khin Mg Tin　4名の報告) 12:30　濱島団長と村田団員，保健局へ直行。D.G. Dr. U Tin U に面会。打合せ。 13:30　ラングーン新病院訪問。同時到着の江見専門家を紹介する。 15:00　医学研究局へ。 午前の研究報告に対する質問。討論を続ける。 18:15　Col. U Kyi Maung （前々保健大臣）を表敬訪問。
20	水	06:45　Meeting （2） 07:30　京大へ出発するビルマ研究医4名 (Dr. Ohn Khine, Dr. Kyaw La, Dr. Myint Soe, Dr. Myint Myint) の訪問，挨拶を受ける。 09:30　医学研究局で，新プロジェクト内容の討議。 14:00　医学教育局訪問。Dr. Pe Thein局長に面会。 14:30　新ラングーン教育病院へ。
21	木	06:45　Meeting （3） 09:00　Hotel出発。大使館へ。 09:40　大使館新田参事館と団員全員保健大臣 U Tun Wai 氏と面会のため Ministry へ行く。 10:00～11:40 長時間にわたり U Tun Wai 保健大臣，U Tun Hla Pru 副大臣，DG. Dr. Aung Than Ba Tu, DG. Dr. U Tin U, DG. Dr. Pe Thein　と会談。 13:00～15:00 医学研究局で。 各部局訪問。Personal discussion

月　日	曜日	内　　容
2　21	木	15:00～16:40 新プロジェクトに関連する，各部局研究現状の視察と討議。
22	金	06:45　Meeting　(4) 07:20　Hotel　出発 07:30　医学研究局着 08:00　"WHO/DMR Research Seminar on Recent Studies of Diarrhoeal Diseases" に参加，保健副大臣 U Tun Hla Pru 出席 09:00～12:00 WHO Dr. の研究発表。 中根教授，畑中教授，団長ら討論に参加する。 13:30～17:30 ビルマ研究者による下痢症研究現状の報告。つづいて討論。終了時間1時間オーバー。
23	土	05:00　起　床 05:30　Hotel　出発，空港へ。 06:45　ミンガラドン空港発，マンダレーへ。 09:15　マンダレー空港着 09:40　マンダレー総合病院着 10:00　マンダレー病院でゲストレクチャー。 畑中教授："Molecular Biology of Viral Diseases" 中根教授："Passing through IgG in the gut mucous membrance" 濱島団長："Stem cell replacement in autoimmune disease" 13:15　マンダレー新教育病院予定地視察。 15:20　マンダレー空港発 パガンへ。 パガン泊。
24	日	16:30　パガン空港発 18:00　ラングーン着。

月　日	曜日	内　　容
2　25	月	06:45　Meeting (IV)
		10:00　ラングーン新病院見学
		濱島団長と村田団員はビルマ側教授団と打合せ。
		午　後　団員一同　報告書書き。
		19:00　調査団長招宴（ビルマ保健大臣，同副大臣，塚本大使出席）
26	火	午　前　報告書書き
		14:00～16:00
		医学研究局で継続討議。
27	水	06:45　Meeting (V)
		10:00　医学研究局で最終討議。
		午　後　報告内容検討および報告書用原稿作成
28	木	06:45　Meeting (VI)
		08:45　第一医学校学長 Prof. Hla Myint 訪問
		10:30　大使館報告
		16:25　Rangoon　空港発 TG305
		18:00　Bangkok　着
		Indra Reagent 泊
3　1	金	08:45　Hotel 出発(Bangkok)
		10:45　Bangkok発 Cathay CX 750
		20:15　成田空港着

Ⅱ－3　関係者氏名一覧

1)　日本大使館

塚　本　政　雄　　大使
新　田　　宏　　参事官
菊　川　晴　博　　一等書記官
篠　浦　　烈　　ＪＩＣＡ所長
高　嶋　俊　政　　ＪＩＣＡ所員

羽根田　　敏　　医務官

2) 国家計画財務省

U Antt Kyaw　Director General, FERD

3) 保　健　省

U Tun Wai	保健大臣
Col. Dr. Tun Hla Pru	保健副大臣
Dr. U Aung Than Ba Tu	Director General, Dpt. of Medical Research
Dr. U Tin U	Director General, Dpt. of Health
Dr. U Pe Thein	Director General, Dpt. of Medical Education
Dr. U Khin Maung Tin	Director, Dpt. of Med. Research
Dr. U Kywe Thein	Deputy Director, " "
Dr. Daw Mi Mi Khin	" " "

BACTERIOLOGY RESEARCH DIVISION	- Head	- Dr. Daw 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. U Khin Maung U
		- Dr. Daw Nyunt Nyunt Wai
		- U Aye Kyaw
		- Dr. U Myo Khin
		- U Tin Oo
EXPERIMENTAL MEDICINE DIVISION	- Head	- Dr. U Hla Myint
		- Dr. U Khin Maung Win
		- Dr. Daw Tin Nu Swe
		- U Tun Khin
IMMUNOLOGY RESEARCH DIVISION	- Head	- Dr. U Tun Pe
NUCLEAR MEDICINE DIVISION	- Head	- Dr. U Thein Than
		- U Mg Mg Thwin
PARASITOLOGY RESEARCH DIVISION	- Head	- Dr. U Myint Lwin
		- U Myint Oo

		- Daw Than Saw
		- Dr. Ye Htut
PATHOLOGY RESEARCH DIVISION	- Head	- Dr. Daw Than Than
		- Dr. U Mg Mg Oo
		- U Thet Win (1)
		- Dr. Khin Ei Han
		- Dr. Daw Soe Soe
VIROLOGY RESEARCH DIVISION	- Head	- Dr. U Soe Thein
		- Dr. U Kyaw Moe
		- U Thet Win (2)
		- Dr. May La Lin
Animal House		- U Khin Maung Zaw
Instrumentation	- Head	- U Soe Myint
		- U Myint Soe
		- U Htay Aung

団長招宴出席者

1. ビルマ側

1. H. E. U Tun Wai (Minister-Ministry of Health)
2. H. E. U Tun Hla Pru (Deputy Minister-Ministry of Health)
3. Dr. U Aung Than Batu (D. G. -Dept. of Medical Research)
4. Dr. U Tin Oo (D. G.-Dept. of Health)
5. Dr. U Pe Thein (D. G.-Dept. of Medical Education)
6. U Aung Tin (D. G. -Dept. of Sports + Physical Education)
7. U Set Maung (D.G.-F. E. R. D.)
8. Prof. U Hla Myint (Rector Institute of Medicine I)
9. Dr. U Khin Maung Tin (Director-Dept. of Medical Research)
10. Dr. U Win Maung (Prof. of Aneathesia)
11. Dr. U Maung Maung Lay (Prof. of Surgery)
12. Dr. U Ba Pe (Prof. of Medicine)
13. Dr. U Maung Kyaw (Director-Dept. of Health)
14. Dr. Daw Mi Mi Khin (Deputy Director [Research] Dept. of Medical Research)
15. Dr. U Tun Yi (Head of Radiology Dept.)
16. Dr. U Maung Ko (Head of Pathology Dept.)
17. Dr. Daw May May Yi (Director-Dept. of Medical Research)

18. Dr. U Khin Maung Gyi (Medical Superintendent)
19. Dr. U Thane Toe (Deputy Director [Research] Dept. of Medical Research)
20. Dr. U Soe Thein (Head-Dept. of Medical Research)
21. Capt. U Kyi Soe (P.A. to Deputy Minister-Ministry of Health)
22. U Hla Nyunt (Assistant Director-Dept. of Medical Research)
23. Dr. U Hla Myint (Head-Dept. of Medical Reserach)
24. U Soe Myint (Head-Dept. of Medical Research)
25. U Hla Pe Than (Asst. Director-F. E. R. D.)
26. Dr. U Thein Than (S. R. O. Dept. of Medical Research)
27. U Tin Nyunt (P. A. to Minister-Ministry of Health)
28. Dr. U Tun Pe (Head-Dept. of Medical Research)
29. U Hla Pe (Head-Dept. of Medical Research)
30. U Kyaw Tint(Additional Director-F. E. R. D.)
31. Dr. Daw Tin Aye (Head-Dept. of Medical Research)
32. Dr. Daw Than Than (Head-Dept. of Medical Research)
33. Dr. U Aung Myint (Deputy Director-Dept. of Health)
34. Dr. U Kyaw Thein (Deputy Director [Admin.]-Dept. of Medical Researc Research)
35. Dr. U Thein Maung Myint (Deputy Director [Research] Dept. of Medical Research)

2. 日本大使館

1. Mr. Masao Tsukamoto (Ambassador)
2. Mr. Hiromu Nitta (Counsellor-Embassy of Japan)
3. Mr. Hirayoshi Sakuma (Counsellor-Embassy of Japan)
4. Mr. Haruhiro Kikugawa (First Secretory-Emb. of Japan)
5. Dr. Satoshi Haneda (Medical Attache-Embassy of Japan)
6. Mr. Tadashi Shinoura (Resident Representative of JICA)
7. Mr. Toshimasa Takashima (Resident Representative of JICA)

3. 調 査 団

1. Prof. Yoshihiro Hamashima (Leader-Preliminary Survey Team for D. M. R.)
2. Prof. Masakazu Hatanaka (Preliminary Survey Team for D. M. R.)
3. Prof. Kazuo Nakane (Preliminary Survey Team for D. M. R.)
4. Mr. Tatsuo Imai (Preliminary Survey Team for D. M. R.)
5. Mr. Ryuichi Murata (Preliminary Survey Team for D. M. R.)

Ⅲ. 調査概要と総括

Ⅲ－1 調査目的とその方針

ビルマ国医学研究局生物医学研究センターにおいて前回の感染症プロジェクトのもとに過去4カ年間になされて来た研究成果に対するエバリュエーションが，去る昭和５８年１２月１８日より２７日まで九州大学田中健蔵学長を団長としてなされたが，その報告書の総合評価の最終結論は，「さらに技術協力をより強力に継続することが望ましい」である。

今回の調査団ではこの田中団長の評価判定の結論を背景にして，次の６項目を主要点として調査を行った。

その項目とは次のようである。

1) 前回のプロジェクト終了が昭和５９年（１９８４）4月9日である。その、技協終了日より現在（昭和６０年１９８５，２月１８日）に到るまでの約１０ケ月半の間に，技協なしでどれだけ医学研究局の研究業績が独自の実力で進展なし得ているか。自分自身の手でどれだけ独立してやって来ているか。

2) 彼らにどのような独創研究の実績を示すことが出来るか。

3) ＷＨＯなどの他の支援機関に対する依存性の実体ならびにＤＭＲとＷＨＯとの今後の関連性

4) 新しく要請して来たプロジェクトの技術協力範囲の妥当性

5) 新プロジェクトがビルマ国に必要とされる絶対的条件とその緊急性

6) 妥当なプロジェクト期間

以上の６項目を本調査の主眼とした。

Ⅲ－2 調査団の予め考えた技術協力具体案

要請書の要旨

今回要請して来た内容の主旨は次のように受けとめることが出来る。

このプロジェクトは肝臓および消化管のウイルス性ならびに寄生虫性感染疾患を中心としたもので，ビルマにおける重要課題の１つである。とくに近時，ウイルス感染に伴う乳幼児重症下痢症の激増ならびに non A·non B 肝炎の大流行と増加とそれに伴う肝硬変・肝癌患者の問題，アメーバ赤痢の全国蔓延など現在ビルマ国保健省の抱えている主要テーマの一つである。

但し技術協力を具体的に実施する段となると，協力期間の４カ年という短時日の要請では，これらの大きな問題の＂治療＂の項目にまで立ち入ることは賛成出来ないので，このプロジェクト名の＂研究＂のみに絞って「治療」は省く方が好ましいと思われる。

従って下記の具体案は研究面のみについて立案したものである。

以下の案はすべて医学基礎研究に重点を指向したものである。

＜消化器系ウイルス感染に関する研究＞

1) ウイルス感染の細胞生物学的研究

従来ビルマのウイルス学研究の実績は，トラコーマ病原体，デング一出血熱ウイルス，日本脳炎に関してはかなりのレベルにまで発展して来ているが，消化器系ウイルス感染症の研究に関しては未だ establish されていない。

とくに，消化管粘膜，肝，胆管，膵を中心とした細胞生物学的研究という地道な基礎的研究から技術協力を開始することが，将来のためにもっとも重要な点である。

2) ウイルス感染の免疫病理学的研究

ロータウイルス，各種肝炎ウイルスあるいは内因性レトロウイルスなどの組織細胞内変化を免疫病理学的に研究する。とくに Reoviridae科の新しいロータウイルスの感染（トロウイルス，ブレダウイルス等）の大流行が見られていることから，ビルマにおける乳幼児重篤下痢症の実体をも把握する。

3) ウイルス腫瘍

イ. がん誘発因子機構

食道がん，胃がん，大腸がん，肝がん，膵がんなど消化器主要がんの誘発因子の研究

ロ. がん増殖の分子学的機構の研究

4) 肝炎の病理

ビルマにおけるウイルス肝炎の実体と分類

とくに non A·non B ウイルスの seroconversion の研究

5) 疫学調査

ビルマの消化器系ウイルス感染症ならびに寄生虫感染症の疫学調査

以上の案を中心として今後，具体的に検討を進める予定である。

なお，この技術協力においてはビルマ国における医学研究への貢献は計り知れない程大きいものであるのみならず，わが国研究者にとっても，新しい事実を次々に見出す重要な機会でもあり，研究の展開が大いに期待されるものである。

Ⅲ－3 調査実施

本事前調査は昭和６０年２月１９日より同２月２７日の間に，ビルマ保健省医学研究局カンファレンス・ルームで行われた。

本調査団は全員ラングーン市インヤレークホテル宿泊で，調査期間中は連日，早朝６時４５分より調査打合せミーティングを行い，討議と検討に万全を期した。

2月19日(火)

2月19日9時10分より10時まで，日本大使館に塚本大使表敬訪問ならびに調査の打合せを行う。

午前10時10分よりビルマ保健省医学研究局にて調査開始。まず本調査の基準対象となるビルマ研究者の最近のオリジナルな研究データの報告から始められた。

冒頭，Aung Than Ba Tu 医学研究局長より，研究発表の前に，今回の新しいプロジェクトに関連して，その要点について説明があった。それによると，日本側からの技術協力を仰ぎたい最重点課目として

① 免疫病理学

② 免疫化学

③ Recombinant DNA の技術

の3つの専門領域の進展を計りたい。

また対象とするヒト疾患としては

① non A·non B 肝炎

② 原発性肝癌

③ ロータウイルス感染症

④ アメーバ赤痢症

以上の4疾患に焦点を絞ったプロジェクトを計画していると説明した。

続いて4人のビルマ研究生から最近の研究成果についての発表があった。

① non A·non B 肝炎と妊婦死亡率について

Dr. U Hla Myint (Head, Experimental Medicine Research Division)

② ビルマにおけるロータウイルス感染下痢症について

Dr. U Soe Thein (Head, Virology Research Division)

③ アメーバ赤痢症の現状と診断法

Dr. U Myint Lwin (Head, Parasitology Research Division)

④ Recombinatn DNA technology

Dr. U Khin Maung Tin (Director)

以上の4題が提示されたが，一般にわれわれに提示してくれた彼ら独自の研究データは少く，レビューに流れる傾向にあったがために，団長，中根教授，畑中教授よりかなり厳しい質問が集中的に出され，研究の厳格さを教示した。

午後には1時から3時まで各部局を訪問して individual discussion を行い，その後，午後3時より4時30分まで，われわれ調査団はビルマ側に対して次のような3項目について質問を行い，ビルマ側の考えの返答を要求した。

われわれが示した質問とは，①Unifying concept は何か？その哲学を示せ，②Uniqueness ビルマ研究生はどこにユニークな点があるのか？ ③従来の素晴しい成果の上った研究と今回の新プロジェクトとの関係を示せ。（日本側の意見としては，是非継続して欲しい。）

以上のわれわれの提示した質問に対して，次のような返事がなされた。

DG. RESPONSE TO INITIAL JAPANESE REACTION TO THE PROPOSAL

Unifying Concept

1. There are two themes underlying the Project:- one vertical
one horizontal.

The vertical theme is - Research on Infections of the Alimentary System.

The horizontal theme is - Research capability strengthening, and these two themes may be seen to interact and complement each other.

2. The different components of the vartical theme are:-

- Research on Viral infections of the Liver
- Research on Viral infections of the gut
- Research on parasite (E. hist.) infection of the gut
- Research on parasite infection (E. hist.) of the liver
- Research on bacterial infection of the gut

Because we will deal with viral, parasite and bacterial infection of two organs viz. - gut and liver - different approachs and techniques will have to be used - but the unifying concept for the vertical theme - in INFECTION OF THE ALIMENTARY SYSTEM.

However, if it seems that there are too many components in the vertical theme - we are ready for listen to suggestions to delete or curtail some of them - for eg. - parasitic infections may be deleted.

3. If we analyze the horizontal theme also - we will find that there are 1-2 components viz.

- Development of Recombinant DNA
- Development of Immunopathology.

If there are too many details some may be delectd.

eg. - Pathophysiology of viral diarrhoea.

Uniqueness

Regarding uniqueness:

- NANB Hepatitis (esp. feco-oral type) has been found to be very high in Burma - with big outbreaks occuring in over 20,000 cases - Few countries have recorded such outbreak but of course Burma is not unique.
- India has had such out-breaks
- Hepatocellular Carcinoma is highly prevalent in Burma- but Burma is not unique - Taiwan, China are example of countries in such higher to prevalence.
- Viral diarrhoea and ammoebic dysentery are all fainrly common diseases what are then UNIQUE about this project?

The UNIQUENESS, in my opinion, is the OPPORTUNITY to undertake GOOD SCIENTIFIC COLLABORATIVE RESEARCH BETWEEN Burmese and Japanese Scientists on some unsolved Biomedical Problems.

The Combination of circumstances is UNIQUE. The coming together at the same point in time of different - favommable circumstances is UNIQUE.

- We have ready the Biomedical Research Centre donated by the Japanese Government.
- We have Japanese and Burmese Scientist who have shown that they can work together and produce good scientific work - as exemplified by the previous DMR/JICA - Project.
- We have certain biomedical problems and disease - problems not only of national but also of worldwide scientific interest, problems which, as a scientist, I feel are just ready for breakthrough.

These problems ready for breakthrough are NANB hepatitis and viral diarrhoeas.

To use a military analogy - scientists in the world have made a foothold - established a bridge-head and are poised for a breakout.

Then again with regard to research capability strengthening.

- JICA cooperation during the last 4-5 years have strengthened DMR research capability to the point where we are just about to take-off. We need an additional push to take-off. We would like JICA to give the additional push. We would like to give JICA the opportunity of giving us the addi-

tional necessary push.

"We do not think the elephant should be stuck just at the tail".

CONTINUITY

We agree that there should be continuity.
We believe that there is continuity
If we review the history of DMR/Japan Coorperation -

- The JICA - started in 1974? by helping set up the VIROLOGY LAB and training VIROLOGISTS of DMR - and by establishing the Electron microscope Lab in DMR.

Some work was started on Enteric virus and Arbovirus - with the DMR/JICA Technical Coop: agreement in 1980-1984.

- VIROLOGY was more focussed on DENGUE VIRUSES
- a begining was made in Rotavirus infection
- and considerable work in Bacteiology of Diarrhoea was done.

In the present Cooperative proposal -

The continuity is in ENTERIC VIRUSES -

- To continue more research on Rotavirus and other virus of diarrhoea
- To start research on feco-oral - NANB virus - which after all is an ENTERIC pathogen.

We think also that some leads which arose from the previous DMR/JICA Project on Bacteriology of Diarrhoea should be taken up and pursued.

So we can perceive a continuing thread in the diseases studied.

THEN there is again a continuity in Research capability strengthening.

During the previous project Pathology and Immunology were strengthened and experitise was increased.

In this present project we intend to more strongly emphasize Pathology and Immunology for strengthening.

- Recombinant DNA technology may be taken as a logical development of Immunochemistry or Virological and Bacteriological - Genetics

Then - there will also be continuity in the Burmese Scientist - people who

has gained expertise from the previous Project will be continuing - with the addition of new talent.

We will be capatalizing on the previous expertise.

OPTIONS

One of the important reasons why the present Project was chosen by us for prepasing to JICA was the need for continuity as explained before. There are many diseases which need STUDY in Burma - Snakebite, Malaria, Goitre - However - looking at the diagram where out interests and your interests and the need and expertise coincide them the AREA chosen for the present Project seems best.

LASTLY

We have made our proposal -

We have given our reasons -

We are willing to listen to SUGGESTION and COUNTER - PROPOSAL within the terms of Reference of your team.

2月20日(水)

9時30分より医学研究局において，新プロジェクト関連部局の研究者による研究発表がなされた。

① ビルマにおける肝炎に対する免疫生化学的診断法について

U Hla Pe (Head, Biochemistry Research Division)

② マラリア脳症の病理

Dr. Than Than (Head, Pathology Research Division)

③ ビルマにおける下痢症対策

Dr. Khin Maung U (Head, Clinical Research Division)

以上の3題についても真剣に十分な討論がなされ，ビルマ研究生の真面目な研究態度に感服させられる点が多かった。

2月21日(木)

午前10時より調査団全員，保健大臣 U Tun Wai 氏，保健副大臣 U Tun Hla Pru 氏を表敬訪問，医学研究局における新しいプロジェクトに関する意見を率直に交換した。約2時間の

長きにわたり熱心な話し合いがなされた。日本大使館から新田参事館が同行された。

午後1時よりは医学研究局におけるこれまでの下痢症に関する研究成果を中心として報告を受けた。その業績は次のようである。

RESEARCH ON BACTERIOLOGY OF ACUTE DIARRHOEA

1. Research Objectives/activities

(A) Biochemistry of Toxins produced by enteric pathogens

i) Biochemical characterization of Shigella toxins.

ii) Development of methods for the identification of toxins produced by diarrhoegenic Shigella.

iii) Further work on the biochemical properties of Escherichia coli heat labile and heat stable toxins.

(B) Serotyping of Escherichia coli

i) Serotyping of 'O' and 'H' of isolates of E. coli prevalent in Burma.

ii) To serotype untypable serotypes from Burma.

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

2. Biochemistry of Toxins produced by Enteric Pathogens

(A) i) Biochemical characterization of Shigella Toxins.

The elucidation of the cellular mechanisms of action of the bacterial protein toxins remains a complex problem. Cytotoxic, neurtoxic and enterotoxic activities of Shigella toxin have all been demonstrated in shigella culture media and bacterial lysates. A number of workers have studied the effects of partially purified shiga toxin in HeLa cell system for cytotoxic property. In contrast, a number of cell lins 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 endycytic) 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 - 7000 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 death is under active investigation.

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 diarrhoeal 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 shigella 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 Objectives and Rational

There is now much evidence that most cases of acute diarrhoea are caused by infection with certain toxigenic strains of E. coli, LT or ST. Although very little direct information is available on the internalization of LT by target cells, presumably the mechanism is essentially the same as that of cholera toxin. Furthermore, no evidence was obtained of toxin-catalyzed 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 differences in their binding specificities. Most notable is the much higher binding effinity 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-ginding domain (s) on the B chains of these two toxins.

E. coli ST toxin is quite small (approx. 2000 MW) and apparently elicits its enterotoxic response by stimulating guan late 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.

(B) Serotyping of Escherichia coli

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

ii) To serotype untypable serotypes from Burma

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

Objectives and Rational

Enterotoxigenic E. colo 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

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

(or)

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

List of research on Diarrhoeal Diseases in Burma

Completed Research Projects

Subjects	Institution where research was conducted
1. Research on bacterial enteric diseases and application of its results for control of these diseases	DMR/Bact. Res. Div.
2. Multicentre hospital based control study of the aetiology of diarrhoea in children under 3 years in different geographic regions. (Bacteriological identification)	DMR/Bact. Res. Div.
3. Clinical trial of Becozamycin in acute bacterial diarrhoea and acute bacillary dysentry.	DMR/Bact. Res. Div.
4. Laboratory aspect of endotoxaemia and septicaemia in acute Neonatal diarrhoea	DMR/Bact & Clinical Res. Div.
5. Isolation and identification of campylobacter jejuni from children intestines	DMR/Bact. Res. Div.
6. Clinical trial of chlopromazine in acute diarrhoea in children	DMR/Clin. Res. Div. & DHS/Infect. Dis. Hosp.
7. Clinical trial of glucose versus sucrose ORS in acute diarrhoea	- do -
8. Clinical trial of Incomplete Formula Oral Rehydration Therapy	- do -
9. Clinical trial bicozamycin, tetracycline & Placebo in acute watery diarrhoea in adults	- do -
10. Clinical trial of Berberine, tetracycline & Placebo in acute diarrhoea	- do -
11. Clinical trial of high dose berberine, tetracycline and placebo in acute diarrhoea	- do -
12. Effect of boiled rice feeding during acute diarrhoea on clinical outcome	DMR/Clin. Res. Div. & DHS/Inf. Dis. Hosp.
13. Effect of breast feeding during childhood cholera on clinical outcome	- do -
14. Biochemistry of blood & stools of neonates with acute diarrhoea	DMR/Clin. Res. Div. & DHS/Special care Baby Unit, C. W. H.
15. Endotoxaemia and septicaemia in Neonate gastroenteritis	- do -

Subjects	Institution where research was conducted
16. Effect of Soya bean milk on clinical outcome from acute diarrhoea	DMR/Clin. Res. Div & DHS/Rgn. Childn. Hosp.
17. Effect of Alum. tetraborax on clinical outcome from acute diarrhoea	- do -
18. Mortality and morbidity from acute diarrhoea in children admitted to Rangoon Children Hospital	DMR/M. Med. So. (Pediaetrics) Thesis at Clinical Res. Div.
19. A study on aetiology of Neonatal Gastroenteritis at neonatal unit Rangoon Children Hospital & Special care Baby Unit, Central Women Hospital.	DMR/M. Med. So. (Pediaetrics) Thesis at Clinical Res. Div.
20. Multicentre hospital based control study of the aetiology of diarrhoea in the first 3 years of life	WHO Clin. Res. Div. DMR {Bact. Res. Div. Vir. Res. Div. & DHS Rgn. Childrn. Hosp
21. Clinico epidemiological study of Residual deaths from acute childhood diarrhoea	DMR/Clin. Res. Div. & DHS/Rgn. Childn. Hosp.
22. Morphology and cytokenetics of small intestinal epithelium in response to Enterotoxigenic E. coli stable toxin in suckling wistar rats	DMR/Clin. Res. Div.
23. Effect of berberine on toxic secretory response to Enterotoxigenic E. coli. stable toxin in suckling rats	- do -
24. Change in blood eosinophil counts during and after recovery from cholera	- do -
25. Oral rehydration therapy in the home by village mothers in Burma	DMR/Clin. Res. Div.
26. Aetiology and epidemiology of acute diarrhoea in Burma-Urban Community	- do -
27. Aetiology and Epidemiology of acute diarrhoea in Burma II- Rural Community	- do -
28. Epidemiology of acute diarrhoea in childhood.	DMR/Epid. Rs. Div.
29. Prospects of prevention of acute diarrhoea	- do -
30. Knowledge and practice in relation to acute diarrhoea in a rural community	- do -
31. Epidemiological model of acute bacterial and viral diarrhoeal diseases	- do -

	Subjects	Institution where research was conducted
32.	Some host and environmental risk factors of acute diarrhoea in an urban community Burma	DMR/Epid. Res. Div.
33.	Clinical trial of chlorpromazine in acute diarrhoea	DHS/N. OKK. Hosp.
34.	A KAP study relating to oral rehydration Therapy at Children Ward NOGH	- do -
35.	Early and Residual deaths in acute diarrhoea at Children Ward NOGH	- do -
36.	Multicentre hospital based central study of the aetiology of diarrhoea in children under 3 years in different geographical regions. (Virological identification)	DMR/Virus Res. Div.
37.	Epidemiology and aetiology of acute diarrhoea in Burma-Urban Community (Virological identification)	- do -
38.	Epidemiology and aetiology of acute diarrhoea in Burma II-Rural Community (Virological idenfication)	DMR/Virus Res. Div.
39.	Preliminary study of knowledge, Attitude, treatment and management of diarrhoea in the Rangoon Community	DHS/C. E. U.

Research Projects which are in progress

	Topics	Institution where research was conducted
1.	The application of microbial genetics to the study of pathogenesis of Infant diarrhoea in Rangoon	USAID/ DMR/Bact. Res. Div.
2.	Role of housefly in disemination of enteric bacterial pathogenesis	- do -
3.	Mechanism and action of LT toxin of ETEC	- do -
4.	Enterotoxin production of *E. coli.* (ETEC-ve) by vero and LLC MK_2 cell line	DMR/Bact. REs. Div.
5.	Study of enteroinvasive properties of *Escherichia coli* and *Shigella* by Hep-2 cell assay	- do -

	Topics	Institution where research was conducted
6.	Role of flies in carriage of enteric bacterial pathogens	DMR/Bact. Res. Div.
7.	Clinical trial of Glycine-ORS in Neonatal Gastroenteritis.	DMR/Clin. Res. Div. & DHS/Special care baby unit. C. W. H.
8.	Clinical and pathophysiological determinants of Residual deaths from acute diarrhoea	DMR/Clin. Res. Div. & DHS/RCH
9.	Impact of monitoring and standardized on mortality and morbidity from acute diarrhoea in children	- do -
10.	Changes in intestinal amino acid absorptive transport mechanisms in response to toxins of Vibrio cholerae and enterotoxigenic E. coli in Wistar rats and in jejunal biopsy specimens	DMR/Clin. Res. Div.
11.	Amino acid absorption during cholera	DMR/Clin. Res. Div. & DHS/Inf. Dis. Hosp.
12.	Efficacy of solution used for early home oral dehydration therapy (Human Volunteers Study)	- do -
13.	Changes in cyclic AMP levels of body fluids in patients with cholera.	DMR/Clin. Res. Div.
14.	Endotoxaemia in cluture filtrates of stable toxin and labile toxin producing enterotoxigenic E. coli.	- do -
15.	Enterocyte calmodulin system in response to toxin of Vibro cholerae and Enterotoxigenic E. coli	- do -
16.	Early home oral regulation therapy in a village community	- do -
17.	Incidence of diarrhoea and feeding practices during diarrhoea queried during nationwide	DMR/Nutr. Res. Div. UNICEF assisted
18.	Salmonellosis in fowls	DHS/Nat. Helth. Lab. (Bacteriology Sect.)
19.	Isolation of salmonella from duodenal aspirater	DHS/RHG & NHL Bact. Sect.
20.	Detection of enteric bacterial pathogenesis in colitis	- do -

Topics	Institution where research was conducted
21. Surveillance of diarrhoea Surveillance of cholera	DHS/CEU and NHL Bact. Sect.
22. Effect of appropriate diets during acute diarrhoea	DHS/N. OKK. Hosp.
23. Study of knowledge, Attitude, treatment and management of diarrhoea in Mandalay and Moulmein	DHS/CEU.

Research projects at the proposal stage

Topics	Institution where research was conducted
1. Food and water contamination as possible risk factors for the seasonal increase in acute diarrhoea in Rangoon, Burma	DMR/Epid. Res. Div. (WHO)
2. Community based prospetive study of rotavirus diarrhoea in children under 2 years of age in Rangoon	DMR/Vir. Res. Div. (WHO)

2月22日(金)

WHO/DMR シンポジウム参加。

このシンポジウムに参加したWHOのDr.は，Dr. N. Pierce (ジョンス・ホプキンス大)，Dr. Holmgren (スエーデン)，Dr. Howe (London)，Dr. Takeda (Tokyo)，Dr. Trabulsi (Brazil)で夫々の領域の講演がなされたが，何れも極めて陳腐かつレビュー的報告ばかりで得る処がなかった。ただ Takeda氏のみが独自のオリジナルの発表がなされたのが何よりの救いであった。

2月23日(土)　マンダレー行き

2月24日(日)　パガン旅行

2月25日(月)

団員全員，新病院の見学

午後　　報告書　打合せ

2月26日(火)

午前　　報告書　書き

午後　　医学研究局で新プロジェクト案の最終討論

2月27日(水)

10:00 ～ 12:00

最後の"まとめ"の討論

オンタンバツー局長室にて。

2月28日(木)

9:30　　大使に帰国挨拶

16:25　ラングーン発　　TG305

18:00　バンコック着　　Indra Reagent 泊

3月1日(金)

11:25　バンコック発　　CX750

III－4　両国チームの新プロジェクトに対する討論の一致点

今回の調査によりわが調査団とビルマ側研究局スタッフとの間で交した討論内容をまとめてみると次のようになる。

① 新規要請プロジェクトのタイトルは "Research on treatment of infectious diseases of the alimentary system" とあるが日本側としてはこの中のtreatmentという字は省きたいがどうか；

② ビルマ側はこの新プロジェクトの研究領域内容を，

a. Research on NANB virus(es) and NANB hepatitis

b. Research on Primary hepatoma

c. Research on Rotavirus and other diarrhoea-associated viruses and infec-

tions caused by these agents.

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

とあるが，この内の ⓑ Primary hepatoma は，ⓐ の hepatitis virus ときってもきれない深い縁があって切り離せないことから，ⓑは抹消した方がよい。hepatoma は必然的にⓐの項目の中に含まれるものである。

また最後にⓔとして Research on Bacteriology of diarrhoea を入れるのが望ましい。

③ タイトルにある alimentary system に膵臓が入るのか入らないのか？

④ non A·non B 肝炎の研究要請に重点をおいているが，調査団としてはいま一つ，ビルマ側が一体どういう点を中心として日本の技術協力を求めているのか，かつそれがビルマ独特の，他の国とは異なったユニークな研究テーマとは一体何なのか，具体的に示して欲しい。

⑤ 日本側としては，この新しいプロジェクトの中には，従来，過去約２０年間にこの研究局が素晴しい成果を挙げて来たデング一熱ウイルスの研究や，病原性大腸菌毒素抽出の研究などを是非継続して進展させて頂きたいのだがその点についてどう考えるか？

⑥ Recombinant DNA technology の開発を要請しているが，この新プロジェクトの開始早々では時期尚早と思われるが，その点をどう思うか？

⑦ 日本側としては，この新プロジェクトを遂行，成功させるためには次のような人員配置が必要と考えられ，かつこれを推薦するがビルマ側の意見はどうであるか？

日本側の推薦案（新プロジェクト要員として）

実験医学部門	医　師	２名
	技術員	４名
寄生虫部門	科学者	１名
	技術員	１名
微生物学部門	科学者	３名
	技術員	４名
ウイルス学部門	科学者	１名
	技術員	２名
病理学部門	科学者	１名
	技術員	３名
免疫学部門	科学者	１名
	技術員	２名
臨床研究部門	科学者	１名
	技術員	１名

以上，少くとも，本プロジェクト遂行のためには研究者１１名，技術員２２名を必要とするものと，日本側調査団は考えた。

⑧ 新プロジェクトの技術協力期間は何年を要求するか？

これに対してビルマ側からは下記のような返答があった。

DMR/JICA DISCUSSION - 19 FEBRUARY TO 27 FEBRUARY 1985

MEMORANDUM

During the discussions the following general understanding was reached regarding the scope of research to be undertaken under the new project, if and when finally agreed upon by the two governments.

1. The aim of the project would be in general accordance with those stated on page 6 of DMR's proposal transmitted to JICA Preliminary Survey Team on 19 February 1985, except that all specific references to Primary hepatoma would be deleted.

   Thus "Primary hepatoma" would be deleted from line 2 paragraph 2 page 6 (and) Research Area 2 would be deleted (and) Research Area 1 will have "and its sequelae" added after "hepatitis ..... ".

   It was agreed that Primary hepatoma would be studied only in relation to and as a sequelae of hepatitis, more especially non-A non-B hepatities.

2. It was generally understood that although the title of the project mentions the treatment of infectious diseases of the Alimentary system, major emphasis of the new projects would be on the underlying factors which would make better treatment eventually available for Alimentary diseases.

3. It was understood that although the term Alimentary system is used, research under the new project will not involve the pancreas but would be mainly confined to the gut and liver.

4. Regarding research on non-A and non-B hepatitis it was understood that such research should proceed towards identification and definitely proving that the putative isolated virus is the caustive agent. This would involve animal studies which would take place either at DMR (Burma) or in Japan. This matter would need further detailed discussions on train-

ing and upgrading of the Animal Services at DMR.

5. It was understood that some new research leads which arose from the previous DMR/JICA technical cooperation project which terminated in April 1984 may be followed up during the present project, especially those concerning some aspects of the microbiological agents of diarrhoea. Some of the research leads that should be followed up regarding the bacteriological agents of diarrhoea were transmitted to the JICA team by the DMR as an addendum to DMR's proposals transmitted on the 19 February.

6. It was generally agreed that Recombinant DNA techniques would be developed in DMR under the new project. It was mentioned that this new technique may not be necessary at the onset of the project and that training would be required for the necessary techniques and methods to be made available at the DMR.

7. The JICA team gave suggestions as to the personnel that may be needed for the new project and DGMR agreed to take it into consideration having due regard to the overall availability of manpower at DMR and DMR's commitments to its other research programmes.

Ⅳ. 団員調査報告

Ⅳ－1　DMR／JICA による新計画〝消化系感染疾患の治療の研究〟に関する調査報告

団　員　京都大学ウイルス研究所教授

畑　中　正　一

JICAとDMRによる前計画〝細菌性腸疾患と主なアーボウィルス病の研究〟は1980年に開始され，1984年に終了している。この間の成果としては大腸菌由来の毒素（トキシン）の分離と生化学的研究は注目に値する。他の消化系感染疾患の研究は必ずしも期待された成果を得ているとは思われない。特に細菌性及びアメーバ性赤痢の細菌学的及び寄生虫学的研究に力を注ぐ必要がある。上記に関する研究施設はほゞ完備しており，この分野での研究員の質的向上を期待するものである。

アーボウイルス病では特にデング出血熱の研究が一応の成果をあげている。患者より得られた野生のデングウイルスを蚊に接種することにより，ウイルスの分離と固定が可能になっている。蚊体内でデングウイルスが卵巣内卵子に体内感染することを見出しており，予防対策上この知見は重要である。アーボウイルス感染症に対する研究に必要とする設備はほぼ完備しており，研究員もこの分野での研究能力は充分備えている。但し研究成果は前計画で予定されたようには進んでいない。

1980年から1984年までのJICAとDMRによる前計画は主として細菌部門とウイルス部門が中心となって研究が推進されたが，国際的に評価されている専門誌への論文発表は極めて限られている。DMRが自主的に上記計画を継続して真に意味のある研究成果を求める積極的な研究体制をつくることを切望する。

上述したようなDMRの研究設備と内容及び研究陣容，研究成果の評価基盤の上にたって新しいDMRとJICA計画について考察する。

DMR／JICAの新計画に対する評価

消化器系感染疾患の治療の研究はビルマ国民にとって現在切実な問題である。肝臓ではA，B，非A非B肝炎ウイルス，腸ではロタウイルスを中心とした下痢発生に関係する諸ウイルス，細菌，アメーバによる感染の研究が必要となる。新計画では消化器官の中でも特に肝臓と腸の感染疾患の研究に重点を置いているのは妥当である。新計画による研究成果はビルマのこれら諸感染に基く疾患の診断，治療及び予防対策に確固たる医学的基礎を与えるものと期待できる。

新計画では非A非B肝炎ウイルスの輸血によらない，経口，糞便に基因する感染経路と〝病原ウイルス〟の分離と同定に主眼をおいている。現在，日本を含む世界の研究施設で輸血による非A非B肝炎ウイルスの研究が行われているが，未だに確固とした病原ウイルスの分離同定の確定したものは報告されていない。ビルマ国民に経口，糞便により伝染する病原体はA型又はB型ウイルスでないもので肝炎を惹起するものがある，と云う認識以上の証左はない。この肝炎病原体を分離，同定する研究は従って重要であり緊急である。このために研究設備を新しく補足充実する必要がある。ウイルス，細菌，寄生虫免疫，実験及び臨床医学の研究員を中心にして抱括的に病原体及び病因を研究する強力な研究陣容を結成することが重要である。同様

同様のことは日本側の指導陣容に関しても云える。非A非B肝炎ウイルスの専門家と共に微生物学，基礎，臨床医学の抱括的な専門家群による基礎的指導がこの計画遂行に必要である。原発肝がんの研究はこの新計画には重要性，緊急性，特殊性においてビルマでは不必要である。

下痢症を伴う病原体の研究はビルマ国民にとって現在，医療衛生予防の観点から特に重点研究計画に入れられるべき項目であり，新計画は至当である。

患者から直接病原体を分離して研究する必要があり，DMR・JICA と当該協力病院との密接な連繋が必要である。この研究には現存するDMRの研究員が一応の必要条件を満しているが，更に日本側の支援による研究員の資質向上が新計画の達成に望ましい。

結論としてこの新計画の推進を強く支持する。

昭和60年2月27日

畑　中　正　一

Ⅳ−2　調　査　報　告

団　員　　東海大学医学部教授

中　根　一　穂

今回のビルマ国立医学研究所からＪＩＣＡに依頼された研究課題はビルマの国情に良く合った問題である事が重要点である。

研究課題は生物学的に感染因子を分類すると

1. ウイルス
2. バクテリア
3. 寄生虫

になり，各々でビルマで特に多い消化器系感染症病原をとりあげている。ウイルス系では最近A型，B型肝炎を其の他の感染性肝炎の区別が出来るようになったため，ビルマには non A·non B型肝炎の流行が時々あり，計 20,000 にまでになっていることが明解にされた。このビルマにおける nonA·nonB 肝炎は何種類の病因に以来するかは不明であるが，他国の例から見てウイル

ス性肝炎であるとビルマ国立医学研究所の研究員は考えている。この点を最初からウイルス性肝炎として病原因子を追求するには今だ時期が少し早すぎると思われる。他の病原因子である可能性を充分考慮に入れて研究計画を建てる事と又この研究の結果得られた因子で動物実験を行って真実性を確かめる必要がある事を指摘した。肝がんに関する研究も同時提案されているがこの問題と消化器系感染とは直接結べない場合があるため，nonA·nonB 肝炎の研究途中で何らか関係があると考えられた場合をのぞいては今回の課題からははずした方が良いと考える。

他のウイルス性消化器感染症としてrotavirus を提案している。この問題はすでにこの研究所にて過去5年にわたり研究を続けて来ている課題でもあり続行する必要があると考えられる。

バクテリア系では1984年に一応終了した研究課題でもあったShigella foxinと E. Coli foxinの研究をさらに深く分子生物的な技術も取り入れて行う事を提案しており，ビルマにおけるこの種の感染症の頻度から考えても続行するのが良いと考えられる。

寄生虫系ではEntamoeba histolyticaの研究を提案している。この問題もビルマにおいて研究を必要とする事であり，今計画では基礎的に分類法の確立から始めていて良いと考えられる。

この多種にわたる病原因の追求とは別に日本との技術協力を求めているのは方法論的な日本人学者による指導であり，病理学的技術とＤＮＡ等の近代技術の導入を希望している。いずれも病原因の探索には必要であり，これらの協力はビルマの医学の進歩に役立つものと考える。

因ってビルマ政府の要請を受け入れても良いと考える。

Ｍ－3　ビルマ消化器系感染症研究プロジェクト事前調査団に参加して

団　員　　京都大学医学部附属病院事務部長

今　井　辰　雄

出発前に国際協力事業団総裁からの委嘱状を受領し，さて私に何が出来るのだろうかと心配になった。それと同時に生れて始めて外国出張を命ぜられ語学を勉強しておけばよかったと後悔した。

しかし出発するからには一つでも多くのことを得ようと思った。出発前に少しでもビルマのことを知っておきたかったので京都の本屋をさがし廻ったがほとんど紹介された書籍はなかった。濱島教授から借用した「ビルマという国・鈴木孝著」によって出張先ビルマのアウトラインを知ることができた。

２月１８日バンコックで一泊ののち出張国であるビルマ・ラングーン市に着いて少しは予想していたが，なんと貧しい国なのだろうかとつくづく思う。広大な土地と資源，人々も勤勉そうなのに……。迎えの車窓から流れる風景や家並はすべて貧しく暗い感じがした。

２月１８日から始まったビルマ国立生物医学研究センターでの先生方のディスカッションの内容は充分理解はできなかったけれど連日暑い中をディスカッションが続けられた。ビルマ側も研

究データを基に熱心に説明され新プロジェクトの構想もほぼまとまりつつあることは喜ばしく感じた。

センター内の研究室も案内してもらった設備された諸機器は順調に動いており研究の成果も上っているようである。ただ動物舎は立派なのに対し種類，頭数が少ないのではないかと思う。

調査も終りに近づいて気がつくとホテルから研究センターまでの道のりで始めは暗く貧しいイメージが少し変ったような気がする。ビルマ人はやさしく，上品でしかし真面目である。街を歩く人々は明るくにこやかであった。しかし色々の話を聞いていて感ずることは少し独立心に欠けているのではないかと思われる。あれだけ古い自動車を修理して使用しているのから考えるともっと研究心があってもよいのではないだろうか。

今月もラングーンの空は快晴である。光化学スモッグもなく，人々は信心深く，木々には美しい花が咲き乱れ，すばらしい国であった。

今回この調査団の一員として出張の機会を与えて下さった関係の方々に厚く感謝すると共にビルマ国の発展を祈念して止まない。

病院の創設について

京都大学医学部附属病院　今井　辰雄

ラングーンに新病院が出来，又マンダレーにも病院創設のプランが進みつつあるので病院の事務部長として意見を申し延べたい。

私は昭和５０年４月富山医科大学附属病院創設準備室総主幹として勤務した。その際経験したことは，

病院の位置

1. 交通の便利はどうか

   職員，患者又は物資の搬入のためには道路が整備されていなければならない。又は容易に整備できる場所でなければならない。

2. 電力の確保はできるか。

   病院にとって電力は必要不可欠のものである。医療機器を始めとしてエレベーター等多くの電力が必要なため。

3. 水の確保はできるか。

   ビルマの場合，水が非常に悪いと聞く。これらを確保するためには井戸を２本堀っておく必要がある。

4. 廃水処理を確実にすること。

   現在のビルマではあまり問題にはならないかも知れないが，将来，種々の問題が出て来そうなのでこの際立派なものを考えた方がよいのではないか。特にビルマでは水が貴重と

思われるので一般廃水の再利用も必要ではないか。ＲＩ関係の廃水は一般のものと分けて処理することとしたい。

5. 要員のトレーニングについて

医師のトレーニングについてはビルマの場合かなり重点をおいていると思われるが，問題は技師である。機器を選定する場合技師と十分打合せが行われるべきでそのためには技師を工事着工と同時，あるいはそれ以前にトレーニングする必要がある。なぜなら開院して実際に機器を操作するのは医師ではなく技師であるからである。

しかも近年の医療機器の多くはコンピューターが組込まれているものが多く，故障しても手が出せない情況である。日本であればメーカーの専門家を呼ぶことも，パーツを持って来てもらうことも容易であるがビルマではそれは望めない。何とか修理をして使用しなければならない。

私は旧制中学４年～５年は学徒動員で愛知県半田市の中島飛行機製作所にいた。「天山」という飛行機の全体組立の一部を担当していたが，その際，予科練出身の操縦士の見習は飛行機組立てが始まるところから２人～３人が配属され，自分達も組立の一部を担当し，組立が完了し，検査に合格するとその飛行機で各基地に飛んでいった。彼等は飛行機が生まれて死ぬまで生活を共にした。そのため日本を遠く離れた各基地で部品も十分なくてもそれを修理し飛行機を飛ばすことができたと思う。

これらの例からして，工学部出身者を開院前にトレーニングする必要がある。

6. 新型の機器の必要性について

現在の医療機器は日進月歩である。しかし，今のビルマ国に最新型がほんとに必要だろうか。未だ時期が早い気がする。それよりももっと簡単で故障してもすぐ直せるもので正確なデータを出すことが先決のような気がする。今や日本国内は臨調旋風が吹きあれて各大学病院とも工夫をこらす時期に来ている。海外の技術協力についても一考を要する問題だと思う。

V. 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

FEBRUARY 18-28, 1985

C O N T E N T S

1. INTRODUCTION

The Biomedical Research Centre was donated by the Janpanese 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 euipped.

Following completion of the Biomedical Research Centre, technical cooperation on " Research into Bacterial Enteric Diseases and Major Arbo-viral Diseases Projects " 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 co-operation.

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 Dpartment 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 Research 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. Adninistration 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 7 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 Medicene 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 peroids 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 studied 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 Rotavirus and other diarrhoea-associated viruses and infections caused by these agents.
3. Research on Entamoeba histolytica and amoebic infections of the gut and liver.

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

Dr. U Hla Myinr

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

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

Outbreaks 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 widerspread 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 cesium 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 a ∿ c), 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 transfected cells appeared to make virion core antigen (HBC Ag) for limited periods.

Several cultures have been established from: patients with primary liver cnacer 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 infections 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 continuous cell line systems). These include cell culture systems that hasıbeen found suitable for cultivation of HAV and HBV such as: primary AGMK cell.

Attempts to propagate in 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 temperatures, 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^{o} cell culture, normal hepatooytes, Kupffer cells (liver macrophages) and cell population of haematopoietic origin and placental tissues will be used.

References

1. P.J. Provast & M.R. Hilleman (1979). Proc. of the Soc. for Exp. Bio & Med., Vol 160(2), 213-221.

2. Wallace R.E. et al. (1973). In Vitro, 8, 333.

3. J.J. Alexander, E.M. Bay, E.W. Geddes, G. Lecatsas, (1976). S. Afri. Med. J. 50, 1124.

4. Aden, D.P. et al. (1979). Nature, 282, 615.

5. A.J. Zuckerman and C.R. Howard (1979). Hepatitis viruses of Man (Academic Press, London.

6. P. Tiollais, P. Charnay, G.N. Vyas. (1981). Science 213, 406.

7. B.B. Knowles, C.C. Howe, D.P. Aden, (1980). Science 209, 497.

8.(a)E.M. Twist, H.F. Clark, D.P. Aden, B.B. Knowles, S.A. Plotkin, (1981). J. Virol. 37, 239.

(b) G.M. MacNab, J.J. Alexander, G. Lecatsas, E.M. Bay, J.M. Urbanowicz, (1976). Br. J. Cancer. 34, 509.

(c) C. Brechot, C. Pourcel, A. Louise, B. Rain, P. Tiollais, (1980). Nature (London) 286, 533.

(d) J.C. Edman, P. Gray, P. Valenzuela, L.B. Rall, W.J. Rutter, (1980). Ibid., P. 535;

(e) E. Tabor et al. (1981). Intervirology 15, 82.

9. Jean-Loup-Romet-le Monne Marry Frances Mc Lane et al.(1983). Hepatitis B virus Infection in Cultured Human Lymphoblastoid Cells. Sc. Vol. 221 No.4611,667-669.

(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

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 conveslesent 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 Ohircu 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, rabbits -- 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.

References

1. Balayan, M.S. et al (1983). Evidence for a Virus in Non-A, Non-B Hepatitis Transmitted via Faecal Oral Route. Intervirology, 20: 23-31.

2. Bradley, D.W., et al (1979). Experimental Infection of Chimpanzees with Antihemophilic (Factor VIII) Materials. J. Med. Virol. 3 : 253-269.

3. Bradley et al (1983). Post transfusion Non-A, Non-B hepatitis: Physicochemical properties of two distinct agents. J. Infect. Dis. 148 : 254-265.

4. Chircu, L.V. et al (1980). Post transfusion Hepatitis : antigen/antibody systems correlated with non-A, Non-B Hepatitis. J. Med. Virol. 6 : 147-151.

5. Dienstag, J.L. (1983). Non-A, Non-B Hepatitis in Hepatobiliary Disease: Current Concepts and Controversies. Chicago. America Association for the study of Liver Diseases. pp 51-65.

6. Gitnick, G. et al (1982). A prospective study with identification of new serologic marker. Gastroenterology 82: 1068

7. Gitnick, G. (1984). Non-A, Non-B Hepatitis. Etiology and clinical course in Annual Review of Medicine. Palo Alto, California. Annual Reviews Inc. pp 265-278.

8. Hla Myint, et al. A clinical and Epidemiological study of an epidemic Non-A, Non-B Hepatitis in Rangoon (In Press).

9. Hopkins, R. et al (1981). Putative markers in non-A, non-B hepatitis research. Lancet 2 : 154.

10. Kane, M.A. et al (1984). Epidemic Non-A, Non-B Hepatitis in Nepal. JAMA 262 : 3140-3145.

11. Mori, Y. et al (1981). Detection of antigen-antibody system associated with non-A, non-B hepatitis. Lancet 2: 98-99.

12. Prince, A.M. (1983). Non-A, Non-B Hepatitis Viruses in Annual Review of Microbiology. Palo Alto, California, Annual Review Inc. pp. 217-232.

13. Shimizu, Y.K. et al (1979). Non-A, Non-B Hepatitis : Ultrastructural evidence for two agents in experimentally infected chimpanzees. Science 205 : 197-200.

14. Shirachi, R. et al. (1978). Hepatitis "C" antigen in Non-A, Non-B post transfusion hepatitis. Lancet 2 : 853-856.

15. Sreenivasan, M.A. et al (1984). Non-A, Non-B Epidemic Hepatitis : Visualization of Virus-like Particles in the Stool by Immuno Electron Microscopy. J. Gen. Virol. 65 : 1005-1007.

16. Suh, D.J. et al (1981). Specificity of an immuno precipitin test for non-A, non-B hepatitis. Lancet 1 : 178-180.

17. Yoshizawa, H. et al (1980). Virus-like particles in a plasma fraction (fibrinogen) and in the circulation of apparently healthy blood donors capable of inducing non-A, non-B hepatitis in humans and chimpanzees. Gastroenterology 79 : 512-520.

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 women was also reported in an epidemic of NANB 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. Assessment of liver regeneration in fulminant hepatic failure:

   measurement of --- serum AFP levels
   --- bile acid conjugation rate
   --- individual clotting factors

4. Determination of biochemical factors in fulminant hepatic failure:

   --- utilization of glucose
   --- serum electrolytes

5. Clinical study in fulminant hepatic failure with viral hepatitis A, B and NANB.
6. Histopathological study of liver in fulminant hepatic failure.

References

Borhanmanesh, F. et al (1973). Viral hepatitis during pregnancy. Gastroenterology 64 : 304-312.

Bradley, D.W., et al (1978). In: Vyas G., et al, eds. Viral hepatitis. Philedelphia. Franklin Institute Press. pp 14-39.

Dienstag, J.L, et al (1979). Lancet 1 : 1265.

Gimson, A.E.S., et al (1981). IgM anti-hepatitis B core and other serological markers in fulminant hepatitis B. Proceedings 16th Meeting of European Association for the Study of the Liver. Lisbon. pp 13.

Gimson, A.E.S., et al (1983). Clinical and prognostic differences in fulminant hepatitis type A, B and NANB. Gut 24 : 1194-1198.

Gitnick, G. (1984). Non-A, Non-B Hepatitis. In Annual Review of Medicine. Palo Alto, California. Annual Reviews Inc. pp 265-278.

Kane, M.A. et al (1984). Epidemic NANB hepatitis in Nepal. JAMA 262 : 3140-3145.

Khuroo, M.S., et al (1981). Incidence and severity of viral hepatitis in pregnancy. Am. J. Med. 70 : 252-255.

Mori, W. et al (1981). The Schwartzmann reaction : a review including clinical manifestations and proposals for a univisceral or single organ third type. Histopathology 5 : 113-125.

Naidu, S.S., et al (1957). Infectious hepatitis in pregnancy during Delhi epidemic. Indian J. Med. Res. 45 (Suppl): 71-76.

Ohori, H. et al (1981). Characterization of Immune Complexes in Sera from Patients with Non-A, Non-B Hepatitis. In Viral Hepatitis 1981 International Symposium. New York City. Franklin Institute Press. pp 331-338.

6. RESEARCH ON PRIMARY HEPATOMA

Research objectives/activities

A. Cloning of alpha foeto-protein gene.

B. Drug-targetting therapy for primary liver cancer in man.

A. Cloning of alpha foeto-protein gene (see also 9 (b)

Production of 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 test based on recombinant DNA technology including ELISA and RIA tests for alpha foeto-protein.
3. Cloning of AFP gene for bulk preparation of anti-AFP globulin conjugates and testing as a possible drug-targetted therapy of primary liver cancer in man.

B. Drug-targetting therapy for primary liver cancer in man

Homing in a cytotoxic drug to target carcinoma hepatic (AFP positive) cells using anti-AFP globulin sensor is a novel way for selective biosurgical therapy for primary liver cancer and has been shown effective in experimental animal. Such study in human patients need be considered. Production of anti-AFP globulins by recombinant technology appears attractive.

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

Dr. U Soe Thein

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 studies 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 studied 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.

References

1. Madeley, C.R. Viruses and diarrhoea. Problems in proving causation. In de la Maza, L., Peterson, E.M. (eds). Medical Virology II. Elsevier Science Publishing Co. Inc., New York: pp. 81-109 (1983).

2. Nkata, S., Chiba, S., Terashima, H., Sakuma, Y., Kogasaka, R., and Nakao, T. Microtitre solid-phase radioimmunoassay for detection of the human calicivirus in stools. Abstr. Working Conference on Babies, Arbovirus including Dengue, Korean Hemorrhogic Fever and Viral Gastroenteritis. The Japan-United States Cooperative Medical Science Program, Tokyo, p. 16 (1982)

3. Uhnoo, I., Wadell, G., Svensson, L. and Johansson, M. E. Importance of Enteric adenovirus 40 and 41 in acute gastroenteritis in young children, J. Clin. Microbiol. 20; 365-372, (1984)

(ii) Molecular Epidemiology of Rotavirus Gastroenteritis

Relevant Background

Molecular techniques have been used recently to characterize rotavirus isolates and to elucidate the epidemiology of rotavirus infections. Rotaviruses have been studied by an analysis of the migration pattern of the 11 genome segments (the RNA electropherotype) (Kalica et al., 1978). Although RNA electropherotyping provide a useful marker to distinguish virus isolates and to monitor virus transmission, the method cannot reliably determine rotavirus antigenic specificities of serotypes or subgroups. The conventional neutralisation tests that detect serotype are tedious and time consuming. Because of their simplicity and specificity molecular cloning techniques with cloned DNA developed by recombinant DNA will yield hybridization conditions that will be useful to distinguish different strains and even serotypes once serotype-specific nucleic acid probes become available. Cloning of the neutralization (or other) genes from all available rotavirus serotypes could serve as a source of cDNA to be used routinely for hybridization analyses alone or in combination with electropherotyping of RNA from field isolates to determine their serotype (or the origin of each genome segment). When conditions to detect serotype-specific probes are optimized, virus serotypes could be analyzed by rapid 'dot hybridization' assays (Flores et al., 1983). The full potential of this approach can be evaluated directly as more information on the nucleic sequence relatedness of rotavirus strains and as a panel of probes from different rotaviruses become available. Since complete homology between target and probe genomes is not essential for positive reactions it is possible that a single probe will be useful to

identify infections with any one of a number of serologically distinct, but sequence-related members of the rotavirus family. Furthermore, with the availability of several probes from the rotavirus family, group, subgroup or serotype assignment should be possible by varying the stringency of the hybridization reactions. This method should complement future strain analysis with monoclonal antibodies and it may be particularly valuable where the rotavirus undergo antigenic diversity or drift, since a change in antigenicity may not be reflected in a major change in nucleic acid sequence.

Work Plan

Objectives

1. To perform restriction mapping and DNA sequencing of the rotavirus gene responsible for neutralization (serotype-specificity) for cloning of serotype-specific gene probes.
2. To analyze virus serotypes of field isolates of rotavirus by rapid ' dot hybridization' assays using the serotype-specific gene probes.

Methods

Biotin-labelled analogs of TTP and UTP will be used as substrates for nucleic acid polymerase in vitro to produce DNA products (Brigati et al., 1983). The cDNA probe will be hybridized 'in situ' to rotavirus dsRNA immobilized on nitrocellulose filters after extraction of faecal samples. Hybridization will be monitored by either fluorescent antibody or enzymatic detection.

References

1. Brigati, DJ, Myerson, D, Leary, JJ, Spalholz, B, Travis, SZ, Fong, CKY, Hsiung, GD, and Ward, DC. Detection of viral genomes in cultured cells and paraffin-embedded tissue sections using biotin-labelled hybridization probes. Virology, 126: 32-50 (1983).

2. Flores, J, Purcell, RH, Perez, I, Wyatt, RG, Boeggeman, E, Sereno, M, White, L, Chanock, RM, and Kapikian, A Z,. A dot hybridization assay for detection of rotavirus. Lancet, i: 555-558 (1983).

3. Kalica, AR, Sereno, MM, Wyatt, RG, Mebus, CA, Chanock, RM, and Kapikian, AZ,. Comparison of human and animal rotavirus strains by gel electrophoresis of viral RNA. Virology, 87: 247-255 (1978).

(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 has been directly labelled with enzyme or by means of avidin which is coupled 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 (Guesdon 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.

References

1.Guesdon, J-L, Ternynck, T, Avrameas, S.,The use of avidin-biotin interaction in immunoenzymatic techniques. J. Histochem. 27: 1131-1139 (1979).

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 studied 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 studied 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.

References

1. Madeley, C.R. Viruses in the stools. J. Clin. Pathol., 32; 1-10 (1979).
2. Melnick, J.L. and Rennick, V. Infectivity titres of enteroviruses as found in human stools. J. Med. Virol., 5, 205-220 (1980).

C. Pathophysiology of enteric virus infections

The enterocyte is the major site of pathophysiological alteration in rotavirus diarrhoea. Establishment of in vitro techniques to study alteration in subcellular structure and biological processes in the enterocyte as a result of rotavirus infection would yield a better insight to the pathophysiological effects of rotavirus infection on intestinal function and its impact on child nutrition and growth.

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

Research objectives/activities — Dr. U Myins Lidin

Development of simple immunodiagnostic tests for amoebiasis

Relevant Background

According to WHO (1981) for the serodiagnosis of amoebiasis, the major tests which are most commenly used include, indirect haemagglutination, (IHA), gel diffusion precipitation, immunofluorescence, counterimmuno-electrophoresis (CIE), immunoelectrophoresis, latex agglutination, complement fixation and enzyme linked immunosorbent assay (ELISA). For seroepidemiological studies, ELISA has been reported to be promising (WHO 1981) and for detection of E. histolytica antigen in faeces, Enzyme Labelled Antibody method was reported to be important diagnostic tool complementing microscopy.

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

Work plan

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

Phase II(a) - To establish in vitro and in vivo assay systems.

In vitro assay system - MDCK cell line culture
- Baby hamster kidney cell line culture
- Chong liver cell line culture

In vivo assay system - Baby hamster
- New born mice

Phase II(b)

Since immunodiagnostic methods such as Indirect Haemagglutination Test (IHA), Indirect Fluorescent Antibody Method (IFAT), Enzyme Linked Immunosorbent Assay (ELISA) and Cross Immune Electrophoresis methods have already been established in the Division for malaria and ascariasis

The following immunodiagnostic methods will be established

(a) Indirect Haemagglutination Test

(b) Direct and Indirect Fluorescent Antibody Method

(c) Enzyme Linked Immunosorbent Assay and

(d) Others.

References

1. AGARWAL, S.S., SHARMA, P., DAS, P., AHMAD, J., DUTTA, G.P. Microenzyme linked immunosorbent assay for serodiagnosis of amoebiasis. Indian Journal of Medical Research (1981, recd 1982) 74 (Aug) 219-225 (En).

2. GILMAN, R., ISLAM, M., PASCHL, S., GOLEBURN, J., & AHMAD, F. (1980). Comparison of conventional and immunoflourescent techniques for the detection of Entamoeba histolytica in rectal biopsies. Gastroenterology, 78 (3), 435-439.

3. WHO (1981) Intestinal protozoan and helminthic infection, technical report series 666.

9. RESEARCH CAPABILITY STRENGTHENING

Some of the activities undertaken under various Research 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, experitise and the facilities for advanced technology especially in the field of Immunopathology. Further development will enable 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 infrastructure and research areas for absorbing advanced biomedical technologies including genetic engineering.

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

1. Cloning AFP gene for bulk prepartation of anti-AFP globilin.

   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. Drug targetting therapy for primary liver cancer.

   Homing in a cytotoxic drug to target carcinoma hepatic (AFP positive) cells using anti-AFP globulin sensor is a novel way for selective biosurgical therapy for primary liver cancer (proven success in experimental studies). Such study in human patients need be considered. Production of anti-AFP globulins by recombinant technology appears attractive.

   - see also 6(B)

3. Cloning and expressing non- A, non-B hepatitis putative virus gene in E. coli for further studies.

   - see also 5(A) iii

Recombinant DNA techniques.

The following techniques in relation to recombinant DNA technology needs to be set up.

1. Plasmid amplification and isolation
2. DNA isolation
3. Restriction digestion

4. Ligation (recombinant DNA preparation)
5. Construction of gene expression libraries (Transformation)
6. Restriction analysis of DNA by gel electrophoresis
7. Analysis of proteins by SDS polyacrylamide gel electrophoresis
8. Southern blotting
9. Western blotting
10. Nick translation for preparation of radio-labelled gene probes
11. Labelling of proteins, including protein-A, by lactoperoxidase and (I^{131}) iodo gene.
12. Gel, ion-exchange and affinity chromatographic methods for isolation of gene products (proteins) and mRNAs
13. Autoradiography
14. DNA sequencing by the chemical method of Maxim and Gilbert
15. The synthesis of DNA oligomers

References

1. UNDP/WORLD BANK/WHO (1984). Genes and parasites: A laboratory manual (Ed. Morel) Fuadacao Oswaldo Cruz - Rio de Janerio, Brazil: Gregoriadis, G. (1977).
2. Gregoriadis, G. (1977) Targeting of drugs. Nature, 265, 407-410.
3. Tsukada, Y., Kato, Y., Umemoto, N., Takeda, Y., Hara, T., and Hirai, H. (1984). An anti-α -fetoprotein antibody-daunorubincin conjugate with a novel poly-L-glutamic acid derivative as intermediate drug carrier. JNCI. 73, 721-728.

C. Infrasturcture support

To facilitate effective and competent biomedical 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.

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 objectives	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 2 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. It 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 Alimentary 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.

CLINICAL RESEARCH

Effect on clinical outcome of breast feeding during acute diarrhoea

KHIN-MAUNG-U, NYUNT-NYUNT-WAI, MYO-KHIN, MU-MU-KHIN, TIN-U, THANE-TOE

Abstract

The effects of oral rehydration fluid alone and of oral rehydration fluid plus breast feeding on the course and outcome of acute diarrhoea were assessed in two groups of 26 children aged under 2 years. Children who continued to be breast fed during treatment with oral rehydration solutions passed significantly fewer diarrhoeal stools. They also passed, on average, a smaller volume of diarrhoeal stools and recovered from diarrhoea sooner after the start of treatment. Their requirement for oral rehydration fluid was significantly reduced.

Breast feeding exerts a beneficial effect on the course and outcome of acute diarrhoea by reducing the number and volume of diarrhoeal stools.

Introduction

Breast feeding has been advocated in many field and oral rehydration therapy projects; mothers are encouraged to continue breast feeding their child during diarrhoea by the World Health Organisation and other international organisations.[1 2] The prevailing practice and belief by mothers and doctors alike is, however, that feeding a child with diarrhoea makes the condition worse. This is not unfounded as an early clinical trial in 1948 comparing feeding a milk formula with fasting during diarrhoea showed an increase in the volume and frequency of diarrhoeal stools (which, being obvious to mothers, makes them think milk feeding exacerbates diarrhoea) yet a beneficial result for the child because of a net retention of nutrients (which is not apparent to mothers).[3 4] Recent clinical trials of different milk formulas during acute diarrhoea showed that feeding milk formula during diarrhoea did not make the clinical outcome from diarrhoea worse.[5 6]

No controlled clinical trial to compare breast feeding with oral rehydration solution alone during the early acute phase of diarrhoea has previously been reported, so data on the effect of breast feeding during diarrhoea are still lacking. The aim of this study was to compare the effect on the clinical outcome from acute diarrhoea in children aged under 2 years of oral rehydration solution alone and breast feeding plus oral rehydration solution during the early stage of acute diarrhoea.

Patients and methods

We studied children aged 6-24 months admitted to the paediatric wards of the Infectious Diseases Hospital for acute watery diarrhoea of less than 48 hours' duration with grade II (moderate or severe) dehydration,[1 2] who had been normally breast fed. Children with a concomitant illness (such as bronchopneumonia, urinary tract infection, clinically evident malnutrition, or shock), bottle fed children, and children who had received antibiotics before admission to hospital were not included in the study. Every child underwent physical examination, and in each case a relevant history was taken. Initial body weight (kg) was taken on admission before starting rehydration treatment. Informed consent from the child's parent or parents was obtained in every case.

Each child entered into the study was allocated by random numbers to receive either oral rehydration solution alone or breast feeding plus oral rehydration solution. Children requiring intravenous treatment were given initial intravenous rehydration until a state of hydration (absence of clinical evidence of dehydration) had been reached (usually within about four hours after admission to hospital); thereafter these children were randomly allocated to receive either of the above regimens. None of these children was given tetracycline or any other antibiotic. Blood for packed cell volume, specific gravity, and assessment of plasma protein, sodium, potassium, chloride, and bicarbonate concentrations was taken from the patients on admission and every morning till discharge from the study. Amounts and types of fluids given by mouth and intravenously were recorded together with outputs of stools, urine, and vomitus every hour and body weights

Clinical Research Division, Department of Medical Research, Ministry of Health, Rangoon, Burma
KHIN-MAUNG-U, MMEDSCI, MACP, head of division and consultant physician
NYUNT-NYUNT-WAI, DCH, MMEDSCI, senior research officer and paediatrician
MYO-KHIN, MB, BS, research officer
THANE-TOE, MB, PHD, deputy director

Infectious Diseases Hospital, Rangoon, Burma
MU-MU-KHIN, MB, DCH, assistant paediatrician
TIN-U, MB, DTM&H, medical superintendent

Correspondence to: Dr Khin-Maung-U.

every six hours. On admission stool swabs were taken for culture of *Vibrio cholera*.

Ethical considerations were emphasised at all phases of the clinical trial. In most instances mothers tended to adhere to old beliefs and preferred to withhold breast feeding during the early acute phase of diarrhoea. Mothers of children allocated to receive oral rehydration solution alone were allowed to continue their practice for up to 24 hours; thereafter they were requested to resume breast feeding their children. Mothers whose children were allocated to receive breast feeding plus oral rehydration solution were told that no worsening of diarrhoea would occur with continued breast feeding during the early acute phase of diarrhoea and were requested to breast feed their children during the first 24 hours and to continue to do so thereafter. All mothers were assured of their prerogative to withdraw from the clinical trial at any time.

STATISTICAL METHODS

Correct trial size was calculated using data from previous studies according to variable of response used:

Purging volume—For children having a total purging volume of 90 ml/kg/patient, with a standard deviation of 50 ml/kg/patient, assuming that for breast feeding to produce a clinically significant deleterious or beneficial effect, it should produce an increase or a reduction in total purging volume by 50%, for a significant p value at 0·05 level and a power of 90%—that is, β or type II error of 0·10—the trial size for each treatment group was calculated to be 26 patients.

Number of diarrhoeal stools—For children producing a mean of 20 diarrhoeal stools per episode, with a standard deviation of seven per episode, assuming that for breast feeding to produce a clinically significant deleterious or beneficial effect, it should produce an increase or a reduction in the number of motions by one third, for a significant p value at 0·05 level and a power of 90% the trial size for each treatment group was calculated to be 23 patients.

Thus, to comply with both calculations, 26 patients were entered into each treatment group, each patient being allocated by random numbers. Results in the two treatment groups were compared using Student's *t* test.

Results

Table I shows that children in the two treatment groups were comparable.

Children who were breast fed had, on average, five fewer motions ($p<0·05$) than those who were not breast fed (table II). They also produced an average of about 250 ml less of diarrhoeal stools and suffered diarrhoea in hospital for two hours less than children who were not breast fed during the first 24 hours (differences not significant). In addition, breast fed children required 550 ml less oral rehydration solution (a reduction of 25%) ($p<0·02$) than those not breast fed during the early acute phase of diarrhoea.

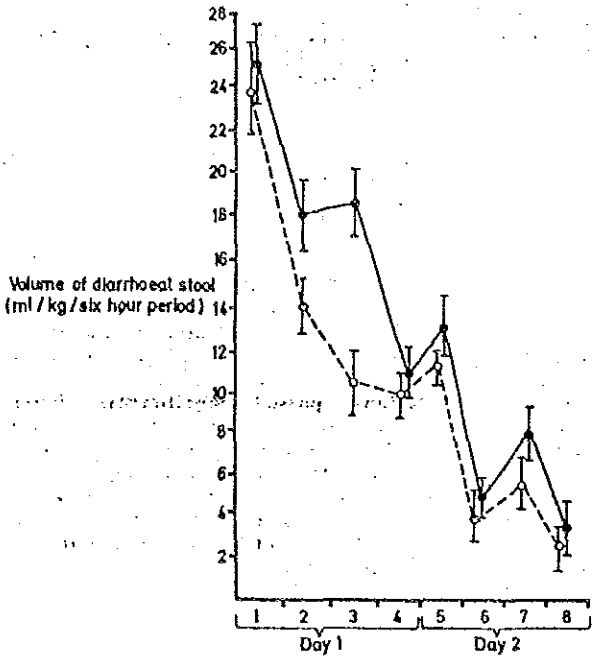
The figure shows that during the initial 24 hours breast fed children produced obviously smaller volumes of diarrhoeal stools per kg body weight per six hour period than children who were not breast fed during the early phase of diarrhoea. In the second 24 hours, when both groups of children were breast fed in addition to receiving oral rehydration treatment, similar patterns of volumes of purging per kg per six hour period were observed.

TABLE I—*Clinical characteristics of children receiving either oral rehydration solution (ORS) alone or ORS plus breast feeding*

Characteristics	Children receiving: ORS alone (n = 26)	ORS plus breast feeding (n = 26)
No of boys	19	15
Mean (SE) age (months)	12·4 (1·1)	10·7 (0·9)
Before admission:		
Mean (SE) duration of diarrhoea (hours)	22·6 (2·7)	24·0 (3·1)
Mean (SE) No of times stools passed	12·7 (1·4)	11·7 (1·3)
On admission:		
No (%) with *Vibrio cholerae* in stools	5 (19·2)	4 (15·4)
No requiring intravenous fluid replacement	8	7
Mean (SE) total intravenous fluid per patient given intravenous treatment	987·5 (268·2)	429·0 (178·4)
Mean (SE) initial body weight (kg)	7·5 (0·3)	7·1 (0·2)
Mean (SE) recovery body weight (kg)	7·7 (0·3)	7·2 (0·2)

TABLE II—*Clinical response by breast fed children with diarrhoea to either oral rehydration solution (ORS) alone or ORS and breast feeding. Figures are means (SE)*

Response variable	Children receiving: ORS alone (n = 26)	ORS plus breast feeding (n = 26)	Significance
Stool output:			
ml/patient	887·4 (116·0)	640·9 (65·5)	$t = 1·8104$, NS
ml/kg/patient	115·8 (14·5)	89·2 (10·0)	$t = 1·5102$, NS
No of times stools passed in hospital	17·4 (2·3)	12·1 (1·1)	$t = 2·0396$, $p < 0·05$
Vomitus volume (ml/patient)	15·2 (8·5)	22·9 (10·9)	$t = 0·5571$, NS
Duration of diarrhoea in hospital (hours)	45·7 (3·9)	43·3 (5·0)	$t = 0·3785$, NS
Total ORS required for rehydration (ml/patient)	2119·2 (192·1)	1570·4 (112·5)	$t = 2·4652$, $p < 0·02$



Changes in mean (SE) volumes of diarrhoeal stools produced every six hours by children receiving either oral rehydration solution (ORS) alone (——●——) or breast feeding plus ORS (- -O- -). Volumes measured on eight occasions at six hour intervals.

Discussion

Although breast feeding during diarrhoea has been advocated by many people,[1 2] no controlled clinical trial to compare breast feeding plus oral rehydration solution with oral rehydration solution alone during the early phase of acute diarrhoea has previously been reported. The findings of the present study suggest that breast feeding during the early acute phase of diarrhoea does not exacerbate the clinical course of diarrhoea and that, in fact, for a lower intake of oral rehydration fluids a larger net absorption of fluids (and nutrients) can be achieved, an effect that may be helpful in obviating the potential risk of hypernatraemia reported in young children given oral rehydration solutions for the treatment of diarrhoea.[3] Products of digestion of breast milk in the children's small intestines, such as amino acids, dipeptides, and hexoses, may also have enhanced the absorption of sodium (and water)[4] thus reducing stool volume and the frequency of diarrhoeal stools.

Other theoretical reasons for continuing breast feeding during diarrhoea also need to be considered. Short term deprivation of nutrients by withholding breast feeding during the early acute phase of diarrhoea is serious as a fasting child loses an estimated 1-2% of his or her body weight daily even in the absence of fluid losses due to diarrhoea.[9] Breast fed children with diarrhoea have been shown to average a total energy intake 35% greater and a protein intake 250% greater than children who are completely weaned.[10] Thus breast feeding not only confers protection against infections including diarrhoea and provides a low cost, highly nutritious source of uncontaminated food but also minimises the reduction in energy and protein consumption during diarrhoea and, as found in this study, exerts a beneficial effect on the clinical course and outcome of acute diarrhoea by reducing the number (and volume) of diarrhoeal stools.

We thank Dr Aung Than Batu for his guidance and criticism and Dr Ba Tun and the nursing staff of the paediatric wards of the Infectious Diseases Hospital for their help with the project. This project was supported in part by a clinical research grant from the Department of Medical Research.

References

1 World Health Organisation. *Treatment and prevention of dehydration in diarrhoeal diseases: a guide for use at the primary level.* Geneva: WHO, 1976.
2 World Health Organisation. *Clinical management of acute diarrhoea. Diarrhoeal Diseases Control Programme. Report of a scientific working group.* Geneva: WHO, 1978.
3 Chung AW. The effect of oral feeding at different levels on the absorption of food stuffs in infantile diarrhoea. *J Pediatr* 1948;33:1-13.
4 Chung AW, Viscorova B. The effect of early feeding versus early oral starvation on the course of infantile diarrhoea. *J Pediatr* 1948;33:14-22.
5 Mahalanabis D. Nitrogen balance during recovery from secretory diarrhoea of cholera in children. *Am J Clin Nutr* 1981;34:1548-51.
6 Braun OH, Sander A. Special milk formulas in the dietary treatment of diarrhoea in infants. *Monatsschr Kinderheilkd* 1981;129:467-71.
7 Cleary TG, Cleary KR, DuPont HL, *et al.* The relationship of oral rehydration solution to hypernatremia in infantile diarrhea. *J Pediatr* 1981;99:739-41.
8 Patra SC, Mahalanabis D, Jalan KN, Sen A, Banerjee P. In search of a super solution: controlled trial of glycine-glucose oral rehydration solution in infantile diarrhoea. *Acta Paediatr Scand* 1984;73:18-21.
9 Rhode JE. Preparing for the next round: convalescent care after acute infection. *Am J Clin Nutr* 1978;31:2258-68.
10 Chen LC, Huq E, Huffman SL. *A prospective study of the risk of the diarrhoeal diseases according to nutritional status of children.* Boston: Harvard University School of Public Health, Department of Population Sciences Report, 1980.

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Effect of long term hormone replacement on plasma prolactin concentrations in women after oophorectomy

D H BARLOW, G H BEASTALL, H I ABDALLA, J ELIAS-JONES, R LINDSAY, D M HART

Abstract

Plasma prolactin concentrations were studied in 88 oophorectomised women who had been receiving mestranol or placebo for three to 11 years. Thirty one of them were also studied under basal conditions and by tests with thyrotrophin releasing hormone. Under basal conditions the mean prolactin concentration was higher in the oestrogen treated group but under non-rested, clinic conditions the difference was lost because of a rise in prolactin value in the placebo group only. Hence the groups showed a different prolactin response to the mild stress of clinic attendance but the same proportionate responsiveness to thyrotrophin releasing hormone.

The data suggest that long term hormone replacement has no significant effect on circulating prolactin concentrations under non-rested, everyday conditions and that the prolactin stimulating effects of minor stress and oestrogen may share a similar mechanism.

Department of Gynaecology, Western Infirmary, Glasgow G11 6NT
D H BARLOW, MD, MRCOG, senior registrar in obstetrics and gynaecology
G H BEASTALL, BSC, PHD, top grade clinical biochemist
H I ABDALLA, MB, MRCOG, registrar in obstetrics and gynaecology
J ELIAS-JONES, MB, MRCOG, registrar in obstetrics and gynaecology
D M HART, MD, FRCOG, consultant in obstetrics and gynaecology

College of Physicians and Surgeons, Columbia University, Columbia
R LINDSAY, PHD, MRCP, professor of clinical medicine

Correspondence to: Dr David H Barlow, Clinical Reader, Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, University of Oxford, Oxford.

Introduction

A large number of women now receive oestrogen preparations on a long term basis. Oestrogen stimulates prolactin release in normal[1] and hypogonadal women,[2] probably by both hypothalamic dopamine suppression and direct action on the lactotroph.[3 4] There has been concern whether women receiving oestrogen preparations long term are at increased risk of developing prolactinoma or breast cancer. In relation to prolactinoma current evidence favours no increased risk in users of oral contraceptives,[5 6] though some workers have mooted such a risk.[7 8]

The question of a relation between use of oral contraceptives and breast cancer has been examined recently.[9] Published work on postmenopausal use of oestrogen and the risk of breast cancer is contradictory, but current data do not suggest a significant increased risk.[10 11] There is a potential for oestrogen treatment to stimulate breast tissue by direct action and by virtue of a chronic increase in circulating prolactin concentrations. The role of prolactin in the induction of breast cancer remains ill defined,[12 13] but there is evidence that prolactin promotes the development and growth of mammary tumours in rodents.[14]

There is limited information on the effect on plasma prolactin of menopausal oestrogen replacement therapy as used in clinical practice.[15 16] This study examines the effect of long term, low dose mestranol on plasma prolactin concentrations and responses to thyrotrophin releasing hormone in a large placebo controlled series originally set up to study oestrogen prophylaxis against postoophorectomy oesteoporosis.[17]

Patients and methods

All patients in the study had undergone hysterectomy and bilateral oophorectomy three months, three years, or six years before entry to the series. Allocation to treatment was randomised and patients took continuous oral mestranol 24 μg daily (17-ethinyloestradiol-3-

VI. TITLES OF RESEARCH PROJECTS

CONDUCTED BY

DEPARTMENT OF MEDICAL RESEARCH

(1980 - 1985)

Bacteriology Research Division

Research projects completed after termination of JICA Project in 1984

1. Isolation and identification of *Campylobacter jejuni* from chicken intestines.
2. Investigation of epidemic fish disease outbreak due to *Aeromonas hydrophilia*.
3. Investigation of dysentery outbreak in Rangoon due to Shiga bacillus.
4. Antagonism of Enterotoxigenic *E. coli* heat labile toxin by mepacrine using CHO cell assay, rabbit ileal loop model and rat ligated loop model.

Research projects in progress after termination of JICA Project in 1984

1. Severity of acute diarrhoea in relation to upper small intestinal pathogen: bacterial components.
2. Role of house fly in dissemination of enteric bacterial pathogens.
3. Biochemistry of *E. coli* toxin.
4. Preliminary and initial set up of preparation of *E. coli* antisera.
5. Initial serotyping work up to *E. coli* isolates collected from community-based studies.

Biochemistry Research Division

Research projects completed during 1980-85

1. Decreased pyridoxal phosphate and kinase levels in iron-deficient liver.
2. Relationship of administered dose to blood venom levels following experimental envenomation by Russell's viper.
3. Anticholineesterase activities of some anthelmintic agents and medicinal plants.
4. Studies on haemoglobinuria produced in rats by the Burmese Russell's viper venom - molecular mechanism and renal damage in RVV envenomation.

Research projects in progress

1. Identification isolation purification and characterization of RVV (Russell's viper venom) toxin and proteins.
2. Biochemical and biophysical studies on the chemically modified RVV toxin and enzymes.
3. Studies on variation in Russell's viper venom composition within species as a function of age and geographical location.
4. Biochemical assessment of renal damage in the human Russell's viper snake-bite victims.

Clinical Research Division

Research projects completed during 1980-85

1. Oral rehydration therapy in the home by village mothers in Burma.
2. Multicentre hospital-based control study of the aetiology of diarrhoea in children under three years in different geographical regions.
3. Iron deficiency anaemia in village children and effect of iron supplementation.
4. Factors associated with Acute Respiratory Infections in children.
5. Clinical trial of Bicozamycin in acute diarrhoea.
6. Effect on clinical outcome of breast-feeding during acute diarrhoea in children.
7. Effect of boiled rice feeding in childhood cholera.
8. Clinical trial of berberine in acute watery diarrhoea.
9. Clinical trial of glucose versus sucrose based oral rehydration solutions in acute diarrhoea.
10. Clinical trial of incomplete formula oral rehydration therapy in acute diarrhoea.
11. Clinico-epidemiological study of residual deaths from acute diarrhoea in children at Rangoon Children's Hospital.
12. Endotoxaemia and septicaemia in neonatal gastroenteritis.
13. Biochemistry of blood and stools in neonatal diarrhoea.
14. Aetiology and socio-epidemiological aspects of neonatal diarrhoea.
15. Morphology and cytokinetics of small intestinal epithelium in response to enterotoxigenic E. coli heat stable (ST) toxin.
16. Changes in cyclic 3'-5'-AMP in stools of cholera patients treated with berberine.

17. Effect of berberine on toxic secretory response to enterotoxigenic *E. coli* heat stable (ST) toxin in suckling Wistar rats.

18. Endotoxaemia in patients with cerebral malaria.

Research projects in progress

1. Changes in intestinal aminoacid absorptive transport mechanisms in response to toxins of enterotoxigenic *E. coli* and *V. cholera* in man and in experimental animals.
2. Clinical and pathophysiological determinants of residual deaths from acute diarrhoea in children at Rangoon Children's Hospital.
3. Clinical trial of Glycine-ORS in neonatal gastroenteritis.
4. Endotoxin in blood and small bowel fluids in neonatal gastroenteritis.
5. Clinical course of diarrhoea in breast-fed children.
6. Early home ORT in acute diarrhoea in village children.
7. Interactions of enteric infections and intestinal absorption: impact on growth and nutrition in children.
8. Improved (Super) ORS with Anti-diarrhoeal Properties: Controlled Clinical Trials in adults and children.
9. Subcellular fractionation studies on enterocytes (human and experimental animal).

Experimental Medicine Research Division

Research projects completed during 1980-85

1. Mother to infant Transmission of Hepatitis B Virus.
2. Placebo controlled double blind trial of Hepatitis B Vaccine in high risk Burmese infants.
3. Post-transfusion Hepatitis in Rangoon General Hospital.
4. Retrospective study of chronic liver diseases in children.

Research projects in progress

1. Efficacy of gamma globulin in the prevention of infection with non-A, non-B hepatitis: trial in pregnant women.
2. Development of ELISA for HBsAg.
3. Laboratory, Epidemiological and clinical investigations of non-A, non-B hepatitis in Burma.

Immunology Research Division

Research projects completed during 1980-85

1. Study of amount of venom injected by *V. russell*.
2. Detection of Russell's viper venom antigen by ELISA technique in human snakebite victims.

Research projects in progress

1. Study of effectiveness of different methods of first aid in Russell's viper bite victims.
2. Study of efficacy of different doses and preparations of ASV in snakebite victims.
3. Development of a rapid immunodiagnostic method for snakebite.
4. Collaborative study of pathophysiology of snakebite with Clinical Research Unit on snakebite.

Nuclear Medicine Research Division

Research projects completed during 1980-85

1. Establishment of radiolabelling of proteins especially radio-iodination of antigen and antibody.
2. Establishment of classical and immunoradiometric (solid phase) sandwich polypeptide proteins especially Russell's viper venom and antivenom.
3. Quantitation of Russell's viper venom and antivenom in the blood of human snake-bite victims by radioimmunoassays.
4. Functional assessment of thyroid by T_3, T_4, TSH measurements in sporadic goitre in low-land Burma.
5. The relationship of administered dose to venom levels in blood following experimental envenomation of Russell's viper (viperae russellii) venom in mice.
6. Plasma clearance time of Russell's viper (viperae russellii) antivenom in human snake-bite victim.
7. Application of solid-phase radioimmunoassay for quantitation of venom and antivenom of Russell's viper (viperae russellii) in tissue fluids.
8. Organ distribution of I^{125}-labelled Russell's viper venom in experimental mice.

Research projects in progress

1. Pharmakokinetic and distribution studies of radiolabelled Russell's viper and antivenom.
2. Establishment of solid-phase radioimmunoassay for malaria and filaria antigens in the host body fluids.

Parasitology Research Division

Research projects completed during 1980-85

1. Comparative study on the micro in-vitro and in-vivo test on its response of P. falciparum to chloroquine in Burma.
2. A study of the relationship between malarial antibody and chloroquine sensitivity in Plasmodium falciparum infection.
3. Correlation studies of chloroquine sensitivity of malaria in in-vitro (macro), in-vivo and in-vitro (macro) test.
4. A preliminary study on the biochemical characterization of Plasmodium falciparum.
5. Establishment and use of Plasmodium berghei. Mouse modle in Burma for the screening of drugs for antimalarial activity.
6. Epidemiology and transmission dynamics of Ascaris lumbricoides in Okpo village, rural Burma.
7. A study on oral quinine therapy in patients with falciparum Malaria.
8. Isoenzyme variation in harvested schizonts of Plasmodium vivax from Burma.
9. In vivo and In vitro response of Plasmodium falciparum to Quinine.
10. In vitro sensitivity of Plasmodium falciparum to Chloroquine, Quinine and Mefloquine.
11. Serological and Parasitological profiles of different malaria endemic areas of Burma.
12. Screening of available Artemesia extracts on rodent malaria.

Research projects in progress

1. To study the way in which the malaria parasite interacts with the host - erythrocyte's pentose phosphate parthway under various conditions such as, G 6 PD deficiency in the presence of oxident and other drugs.
2. Hospital based study of monitoring the change in degree of sensitivity to currently available antimalaria drugs in different areas of Burma.
3. In vivo and In vitro response of Plasmodium falciparum to Sulfadoxine/pyrimethamine.
4. Use of IFAT for the early detection of drug failure cases in a Chemoprophylactic group.
5. Dynamic of Plasmodium falciparum population in an area of persistant malaria transmission.
6. Nutritional status following anti-helminthic treatment in Ascaris infected children.

Pathology Research Division

Research projects completed during 1980-85

1. Role of disseminated intravascular coagulation in the pathogenesis of cerebral malaria.
2. Effect of exchange transfusion on histopathologic lesions in the liver of plasmodium berghei infected mice.

Research projects in progress

1. Histopathological and ultrastructural studies of the brain in cerebral malaria
2. Histopathological and ultrastructural studies of the kidney and other organs in Russell's viper bite victims.
3. Study of hemostatic defects in Russell's viper bite victims.
4. Nephrotoxic action of Russell's viper venom: A study by microdissertion of the nephron.
5. In vitro testing of the action of Russell's viper venom on blood coagulation/fibrinolysis and inhibiter system.

Virology Research Division

Research projects in progress after termination of JICA project in 1984

1. Multicentre Multidisciplinary Epidemiological Study on DHF in Rangoon Community.
2. Localisation of dengue antigen in DHF autopsy materials by IF.
3. Persistence and replication of dengue viruses recovered from different disease manifestations in DHF in $C_{6/36}$ carrier cultures.

EXTRACT FROM RESEARCH FINDINGS

1980 - 1982

DMR/JICA PROJECT

RESEARCH ON MAJOR ARBOVIRAL DISEASES,

BACTERIAL ENTERIC DISEASES,

AND

THE APPLICATION OF ITS RESULT

FOR THE CONTROL OF THESE DISEASES

EXTRACT FROM RESEARCH FINDINGS

1982 - 1983

DMR/JICA PROJECT

RESEARCH ON MAJOR ARBOVIRAL DISEASES,

BACTERIAL ENTERIC DISEASES,

AND

THE APPLICATION OF ITS RESULT

FOR THE CONTROL OF THESE DISEASES

EXTRACT FROM FINAL REPORT,

DMR/JICA PROJECT

RESEARCH ON MAJOR ARBOVIRAL DISEASES,

BACTERIAL ENTERIC DISEASES

AND

THE APPLICATION OF ITS RESULT FOR

THE CONTROL OF THESE DISEASES PROJECT

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

(1980-1984)

1. Aye Aye Myint : (1980-81) M. Sc. (Zoology).
   Rangoon Unversity.
   Title of Thesis: Establishment, Standardization and Application of ELISA in Russell's viper evenomation.
   (Immunology Research Division, DMR)

2. Dr. Phyu Phyu Win: (1980 to date) M. Sc. (Microbiology).
   Institute of Medicine, Mandalay.
   Title of Thesis: A study of Cell-mediated Immunity in Leprosy patients.
   (Immunology Research Division, DMR)

3. Dr. Soe Thein : (1981- to date) M. Med, Sc. (Microbiology).
   Institute of Medicine, Mandalay.
   Title of Thesis: Viability of JE virus in glyceronized mosquitoes and tissues.
   (National Health Laboratory & Virology Research Division, DMR).

4. Khin Mar Aye : (1981-to date) M.Sc. (Zoology).
   Rangoon University.
   Title of Thesis: Virulence and temperature sensitivity of Dengue viruses.
   (Virology Research Division, DMR).

5. Dr. Mya Mya Ohn: (1981-to date) M. Med, Sc. (Paediatrics).
   Institute of Medicine (1), Rangoon.
   Title of Thesis: Neonatal diarrhoea - major etiological agents & mortality patterns.
   (Clinical & Bacteriology Research Division, DMR).

6. Thazin Lay : (1983--1985) M.Sc. (Zoology)
   Rangoon University.
   Title of Thesis: Replication and persistence of dengue virus in Aedes albopictus (C 36) cell culture.
   (Virology Research Division, DMR).

7. Dr. Tin Aung: (1982-1983) M. Med. Sc.

Institute of Medicine I, Rangoon.

Title of Thesis: Effect of E. coli enterotoxin on aminoacid absorption by animal intestinal tissues.