PROCEEDINGS OF THE TECHNICAL COMMITTEE

and

REPORTS OF THE PAKISTAN-SEATO CHOLERA RESEARCH LABORATORY

for the

FISCAL YEAR 1969

(JULY 1, 1968 - JUNE 30, 1969)

VOLUME I

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and
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IN ABSENTIA

With deep regret the PSCRL wishes to express condolence to the many colleagues and friends of Mr. David A. Wraight who passed away suddenly November 4, 1969 of coronary thrombosis. Mr. Wraight served several years on the PSCRL Directing Council representing the Secretary General of SEATO as Deputy. He was a native of New Zealand and served as Secretary of the Navy of New Zealand for 16 years.

HONORS AND AWARDS

In early 1969 Sir James W. Howie, Director, Public Health Laboratory Service, London and member of the PSCRL Technical Committee, was elected President of the British Medical Association. He also was conferred the honorary degree of LL.D. by his alma mater, the University of Aberdeen in Scotland.

Dr. John R. Seal, Chairman of the National Institutes of Health Cholera Advisory Committee who represents the Director of the NIH on the PSCRL Directing Council, was recipient of a Special Award in 1969 given by the Association of Military Surgeons of the United States of America. Dr. Seal also received a citation for scientific leadership in the development of new intramural research programs at the NIH during the first annual award ceremony by NIH held in Bethesda, Maryland, on June 30, 1969.

Dr. Colin MacLeod of the New York University School of Medicine, New York City and Chairman of the PSCRL Technical Committee, received the James D. Bruce Memorial Medal for 1969 awarded by the U.S. College of Physicians for his distinguished service.

On December 12, 1968 during annual meetings of the PSCRL Directing Council and Technical Committee in Dacca, the late Mr. David A. Wraight, Deputy Secretary General of SEATO at the Secretariat in Bangkok, representing the Secretary General, presented SEATO long service certificates and badges to over 60 PSCRL staff who had completed five years of satisfactory service by August 1, 1968. On "SEATO DAY" (September 8, 1969) observances at the PSCRL, Mr. Leslie A. Squires, American Consul General in Dacca, on behalf of the Secretary General of SEATO, presented 57 certificates and badges to PSCRL staff who had completed five years of satisfactory service by August 1, 1969. During

installation of new officers of the PSCRL Staff Welfare Association ceremonies on November 14, 1969, Mr. Eric Griffel, Acting Provincial Director for the U.S. Agency for International Development in Pakistan, on behalf of the Secretary General of SEATO, presented an additional 20 certificates and badges to additional PSCRL staff who had also had five years of satisfactory service by August 1, 1969 but whose receipt of the award had been temporarily delayed by a pending policy review required to amend previous guidelines used to determine eligibility. The Director and management staff were most pleased to amend criteria used previously thus allowing inclusion of the additional members of the staff in the awards. This brings to 137 the total number of PSCRL employees who have received this award through calendar year 1969.

On August 20, 1969 Dr. Phillips presented to Mrs. Constance Miccio on behalf of the U.S. Department of Health, Education and Welfare, a certificate and pin in recognition of ten previous years of service with the U.S. Government. Before being posted to Dacca at the PSCRL in April of 1968 Mrs. Miccio's service included 8-1/2 years with the U.S. Department of State as Foreign Service Staff Officer. She was posted to Dacca 1956-58 and also served in Tokyo, Brussels and Kinshasa (Leopoldville).

The nursing staff of the PSCRL's Dacca ward were given first prize at an exhibition held in Dacca in January 1969 by the Trained Nurses Association of Pakistan, Dacca Branch. The prize was a very fine dictionary, awarded to the staff for designing the best teaching aids. The award is enscribed by the TNA to the nursing staff for "showing simple aids to maintain fluid balance in the case of cholera and acute diarrhea".

On May 9, 1969 the American Ambassador to Pakistan Benjamin Oehlert, Jr., representing the U.S. Department of State, presented to the U.S. Mission staff and their families a citation and medal in recognition of the performance of the American Mission staff and their families during the then-recent "period of disturbances" in Dacca.

On November 5, 1969 Dr. Robert A. Phillips, Director of the PSCRL, was formally notified he had been conferred the 1969 Pakistan Civil Award, Sitara-i-Quad-i-Azam (abrev. S.Q.A.). In replying Dr. Phillips said "I am deeply honored by this award, realizing that it is to the entire staff who have worked with me in our investigations over the last four years. I would like to assure you that I and my staff will continue to make every effort to be worthy of the award."

AGENDA
of the
7TH MEETING OF THE TECHNICAL COMMITTEE
December 1-3, 1969

December 1
Welcoming address, Dr.Robert A.Phillips, Director, PSCRL
Opening address, Dr.Colin M.McLeod, Chairman of the Technical Committee
Tour of facilities and discussion of facilities, Mr. Patrick Talmon, Executive Officer, PSCRL
Group photograph with PSCRL staff
General review of physiology program, Dr.W.Kendrick Hare

December 2
General review of epidemiology program, Dr.W.Henry Mosley
Discussion of epidemiology program, Dr.Alexander Langmuir
Presentation of epidemiology studies by staff
Toxin-toxoid discussions, Dr. John R. Seal and Dr. W.E. van Heyningen
Discussion and presentations of physiology studies, Dr. W.Kendrick Hare and staff

December 3
Continuation of discussion and presentations of physiology studies, Dr. W.Kendrick Hare and staff

December 4
Preparation of Technical Committee report
Visits to Matlab
Various group discussions

(Note: Representatives to the meetings of the Directing Council were invited to attend the meetings of the Technical Committee.)

SCIENTIFIC PROGRAM

FY69

ASSIGNMENT OF CODE NUMBERING SYSTEM

Code numbers are used in the PSCRL computerized financial accounting system for providing cost breakdowns for sundry purposes. The first two digits indicate whether a protocol is a product of one or two divisions, a combined division and Director effort, a combined PSCRL and non-PSCRL effort, or a reimbursable study.

01 - Administrative Division

02 - Maintenance Division

03 - Epidemiology Division

04 - Physiology Division

05 - Combined division study or
combined division - Director study

06 - Training (PSCRL staff)

07 - Combined PSCRL and non-PSCRL study

08 - Reimbursable study

The third and fourth digits indicate the fiscal year in which a protocol is approved and carried out. The fifth and sixth digits list the protocols in numerical sequence for each category.

Example:

08 70 01 -"Demographic studies in rural East Pakistan." This is a reimbursable (08) approved in FY70 (70), the first reimbursable study in the FY70 series.

Example:

05 70 04 - "Inhibition of cholera toxin by aromatic polysulphonic acids." Participation in this joint study by the Director of the PSCRL and by Dr. K.M.S. Aziz (Physiology Division) determined the category (05). The study was approved in FY70 (70), and was the fourth such combined study in the FY70 series.

FY70 protocols enclosed were approved during the period July 1 - November 30, 1969.

PROTOCOL CODE 03 70 01

SECTION I

1969-70 CHOLERA VACCINE FIELD TRIALS AND SEROLOGICAL STUDIES

Wiley H. Mosley
William E. Woodward
K. M. A. Aziz
Ansaruddin Ahmed
A. K. M. A. Chowdhury

SUMMARY

The 1969-70 vaccine field trials will test the efficacy of booster inoculations of the monovalent Ogawa and Inaba vaccines, with particular reference to cross-protection against the heterologous serotype of cholera and the duration of protection induced by the purified Inaba antigen. The populations receiving inoculation over the past three years as a part of the 1966-67 field trials will be observed to determine the cumulative effect of previous injections without further boosting in 1969.

Serological surveys will be carried out to continue to study the relationship of vibriocidal titer to protection. Serological studies of the type specific immunity in cholera, first demonstrated in the 1968-69 field trial, will be carried out by antibody absorption tests to determine the pattern of type specific and group antibodies induced by the various cholera vaccines. In addition, the patterns of type specific and group antibodies in cholera cases which occur in the vaccine recipients will be characterized. Factors contributing to nonspecific vibriocidal antibody titers, such as infections with antigenically-related organisms, including brucella and adjuvant vaccines will be more fully evaluated.

RESEARCH PLAN

Background

The cholera vaccine field trials carried out since 1963 have confirmed the efficacy of personnel immunization but have shown protection to be of short duration. The inoculation schedule used and the age of the recipient have been shown to be significant factors influencing the level of protection achieved. Serological surveys have demonstrated a correlation between vibriocidal antibody titer and protection. The 1968-69 field trial, utilizing monovalent vaccines, has, for the first time, clearly demonstrated that protection is type specific. The Ogawa vaccine did not protect against Inaba cholera; however, it did induce significant Inaba vibriocidal titers which most likely represent antibody against the common group antigen shared by both serotypes. This last observation indicates the need for a more careful study of type specific immunity in cholera, both in terms of characterizing the antibody response to vaccine and to the disease. In addition, factors influencing specific and nonspecific vibriocidal titers must be more carefully evaluated.

Method

The basic procedures for the 1969-70 field trial will follow the patterns established in previous field trials. Census books, identifying all the participants by individual census number and giving their vaccine assignment, are already available. Three random sample serological surveys will be taken from the entire vaccine field trial population by selecting all the vaccine recipients in every thirtieth family. Fingertip blood will be collected in September, 1969, prior to inoculation; in November, 1969, one month after inoculation; and in late December or early January, 1970, three months after inoculation.

Vaccines will be administered by vaccine teams equipped with jet injectors, going from house to house. Recipients of Vaccines F and H will receive booster inoculations of the same vaccines. Recipients of Vaccines G and I will receive booster inoculations of Vaccine I.

Case detection will be maintained by daily house-to-house surveillance by the field staff and all severe diarrheal cases will be treated at the Matlab hospital. Ambulance services will be maintained throughout the entire field trial area by speedboats and autorickshaws. All cases will be identified by vaccine field trial number and confirmation as cholera will be obtained by the usual bacteriological techniques established at PSCRL.

The relative efficacy of the various cholera vaccines will be determined by the number of cases occurring in each of the vaccine groups, as compared to the control group. The protective effect of the vaccines will be related to the vibriocidal titers in the various vaccine groups, as determined by the serological surveys.

Determination of the type specific and group antibodies in sera will be carried out by differential absorption studies with Ogawa and Inaba organisms. This technique will be applied to determine the frequency of type specific antibodies in specimens obtained on serological surveys in normal East Pakistanis, as well as in the recipients of the various field trial vaccines. In addition, absorption studies will be carried out on the admission sera of cholera patients to determine whether the presence of type specific antibody can be specifically related to protection against cholera.

A limited survey of skin test reactivity to brucellergen in the field trial population and a specific study of the effect of inoculations of diphtheria and tetanus toxoids on stimulation of vibriocidal antibodies will be carried out to evaluate some factors which may contribute to nonspecific vibriocidal antibody titers.

Significance

The 1969-70 vaccine field trials are a part of a long-term program to develop an effective cholera vaccine of long duration and to develop laboratory tests that would be suitable for evaluating vaccine effectiveness, without necessitating large-scale field trials. Previous field trials, utilizing bivalent Ogawa and Inaba vaccines, had suggested that the vibriocidal antibody titer of the recipients was a useful measure of vaccine effectiveness;

however, the 1968-69 field trial has revealed that protection is type specific and thus indicates that serological studies in man must be re-evaluated in terms of type specific antibody. The question of type specific immunity is not entirely resolved, however, since the field trials have only shown that the Ogawa vaccine does not protect against Inaba cholera. Booster inoculations of the same vaccine will be given this year in anticipation of an adequate number of Ogawa cases to determine if the Inaba vaccine does or does not cross-protect against Ogawa cholera.

The fuller understanding of type specific immunity in cholera has relevance to the development of a toxoid vaccine. A toxoid field trial is being delayed in part to prepare to toxoid pure enough so that it will not induce bacteriocidal antibody and presumably antibacterial immunity. If antibacterial immunity is type specific, it may be possible to prepare two toxoids, one from an Ogawa and another from an Inaba organism, and in a field trial any protection achieved by the toxoid vaccine to the heterologous serotype of cholera, may be attributed to antitoxic immunity and not to residual antibacterial immunity that may have been induced. More remotely, if it is ultimately found necessary to combine the toxoid with a vaccine inducing antibacterial immunity, the toxoid which would probably be prepared from an organism of only one serotype, would have to be combined with a cell wall antigen from the other serotype.

An evaluation of the duration of immunity produced by the purified Inaba antigen is relevant to a consideration of its incorporation in an adjuvant vaccine. A followup of the children who have received cholera inoculations for three consecutive years, since 1966, will determine if they are beginning to develop any cumulative effect of repeated cholera vaccine in terms of more prolonged protection.

Characterization of factors that may be responsible for nonspecific vibriocidal antibody titers are important in clarifying the interpretation of the relationship of vibriocidal antibody to immunity in the endemic cholera areas.

Facilities Available

The facilities include the Matlab vaccine field trial area, with a defined population under regular house-to-house surveillance, a fully trained field staff, a field hospital with speedboat ambulance service, and other logistical support - jet injectors for the administration of vaccines and laboratories available for the serological, bacteriological and statistical support.

Collaboration

These field trials are being conducted in collaboration with Drs. Margaret Pittman and John Feeley of the Division of Biologics Standards, National Institutes of Health, and Dr. Willard Verwey, Professor of Microbiology, University of Texas Medical Branch, Galveston, who provided the purified Inaba antigen.

Supporting Data

Results of previous field trials and serological studies have been published.

SECTION II

DETAILED BUDGET

		Rupees
Personnel		
Field Surveillance staff		501,214
Matlab Hospital staff		98,510
Admin. and supporting staff (speed boat driver, guards, etc.)		89,793
Bacteriology - 20,000 man hrs. at Rs 5.00		100,000
Immunology - 30,000 man hrs. at Rs 3.00		90,000
Statistical - 800 man hrs. at Rs 4.40		34,200
Consultant		
Dr. Fahimuddin		14,200
Dr. Furniss		6,000
Equipment		
Lyophilizing apparatus	$9,000	43,200
Supplies		
Diphtheria-Tetanus toxoid vaccine	$ 200	960
Spare parts jet guns	$4,000	19,200
Spare parts - speed boats	$5,000	24,000
Serology supplies - complement, glassware, microtiter parts, etc.	$2,000	12,000
Bacteriology supplies - media plates	$5,000	28,000
Lyophilizing supplies	$2,200	10,560
Statistical forms, IBM cards, pencils, etc.		2,000
Matlab Surveillance supplies	$ 800	50,000
Matlab Administrative supplies	$ 300	7,000
Travel		
A. Local		
Fixed traveling allowance - field staff		90,600
Country boat hire		45,600
Differential allowance		33,600
Messenger service		18,000
Petrol transport		4,800
B. Foreign		
Dr. Mosley		7,600

Hospitalization and outpatient		
Diet - Matlab Hospital		20,000
Supplies and medication	$2,500	42,000
Alterations and renovation		
Renovation of Bacteriology Lab.		50,000
Publication costs		
Secretarial		6,000
Reprints, etc.		1,000
Other expenses		
Food for vaccine campaign		22,000
House rent, nurses and speed boat drivers		5,000
Rental, ambulance station		1,500
		1,477,537
Overhead 30%		443,261
Total		1,920,798

(Note: Dollar amounts have been indicated for Equipment and Supplies which have been included in the rupee amounts indicated. The rate of conversion used was Rs. 4.81 for $1 (US), rounded off.)

Addendum to 03-70-01

22 July, 1969

In the budget summary on the protocol for the 1969-70 cholera vaccine field trials, I included Rs 10,000 as food allowance during the vaccine campaign. Last year Rs 65,000 were spent for food allowance for the vaccine campaign and serological survey. As a result, in the proposed Laboratory budget, the amount of Rs 65,000 had been allocated for food allowances for 1969. In drafting the budget for the 1969-70 cholera vaccine field trials, I felt I could reduce this to Rs 10,000 by eliminating food allowance for the serological surveys. After consultation with Mr. Aziz it turns out that this will be too drastic a curtailment of the budget in this category. Therefore, I am requesting that the food allowance for the vaccine campaign and serological surveys be increased by an additional Rs 12.000.

Wiley H. Mosley, M.D.
Head, Epidemiology Division

PROTOCOL CODE 03 70 02

SECTION I

FURTHER STUDIES OF THE EPIDEMIOLOGY OF EL TOR CHOLERA

I. Surveillance of El Tor Cholera in Chittagong

Kenneth J. Bart

SUMMARY

The first documented El Tor cholera epidemic in East Pakistan was studied in Chittagong in 1968. The spectrum of illness, the secondary attack rate and the case to infection ratio delineates the disease caused by El Tor vibrio to be epidemiologically different from classical cholera. The occurrence of cholera in Chittagong will be under daily surveillance by bacteriologic investigation of all patients admitted to hospitals requiring intravenous rehydration. The protocol is designed to define the epidemiologic pattern of El Tor cholera, and to provide an outpost for surveillance to recognize communities suitable for intensive epidemiologic studies.

(Note: Please refer to Protocols E/P15 reported last year and 03-70-07 reported this year.)

RESEARCH PLAN

A. Introduction and Specific Aims

Despite the spread of El Tor cholera through the subcontinent in epidemic form, East Pakistan has remained primarily afflicted with the classical vibrio with only occasional cases of cholera caused by the El Tor vibrio. The first documented epidemic of El Tor cholera occurred in Chittagong in 1968.

Studies performed during 1968-69 in Chittagong when both El Tor and classical cholera occurred simultaneously, provided information about the spectrum of illness, the secondary attack rate, the case to infection ratio and the ability to isolate the vibrio from the environment, delineating disease caused by the El Tor vibrio as distinct epidemiologically from the disease caused by the classical vibrio.

We plan to continue surveillance of the occurrence of El Tor cholera in Chittagong. Through continued surveillance we will be better able to understand the epidemiology of El Tor cholera. The 1968 epidemic may be heralding the spread of El Tor cholera through East Pakistan, as did the first epidemic in Calcutta in 1964. In addition, the occurrence of another outbreak will provide the community for proposed study of inapparent infection which was part of last year's protocol.

B. Method of Procedure

All patients admitted to the 5 hospitals in Chittagong Municipality which accept patients with gastroenteritis requiring intravenous hydration will be visited by a male and a female field assistant daily. Patients admitted during the preceding 24 hours will be questioned for historical and demographic information and a rectal swab culture will be obtained. The rectal swab will be placed immediately into bile peptone.

At the end of the day after all hospitals have been visited, the cultures will be sent via PIA to Dacca daily for processing in the PSCRL bacteriology laboratory.

C. Significance

The epidemiologic differences between El Tor and classical cholera have not been completely defined. The El Tor vibrio has been isolated frequently from many communities since its discovery by Gotschlick (1906) with varying interpretations of its epidemiology. In addition, the variability of the testing defining the biotypes has made epidemiologic interpretation of this early work nearly impossible.

The combination of good methods for biotypic definition and the occurrence of El Tor cholera in East Pakistan will enable continued epidemiologic definition of the El Tor vibrio.

D. Facilities

The bacteriology section provides the diagnostic laboratory for this project.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
Kenneth J. Bart	Research Physician	10	-	-
Mr. N. Zaman	Field Assistant	20	7,308	1,462
Mr. Shafiullah	Field Assistant(Temp)	100	2,400	2,400
Miss Minu Mozumdar	Field Assistant(Temp)	100	2,400	2,400
Bacteriology Lab.				
Equipment	None			
Supplies	Swabs, media plates, etc. for 2,000 rectal swabs processed for V. cholerae.			10,000
	Miscellaneous office equipment and supplies (staples, mimeographed forms, wrapping paper, pens, etc.)			500
Transport	PIA transport of swabs daily Chittagong to Dacca (@Rs 3).			1,500
	PIA transport of media once a week Dacca to Chittagong (@ Rs 6)			500
	PIA return fare to Dacca/ Chittagong every 2 weeks			3,000
	Baby taxi fares in Chittagong between hospitals			3,600

All other expenses	Rent of office	100
	Secretarial	1,000
	Publication	2,000
		28,462
Overhead 30%		8,539
Total		37,001

PROTOCOL CODE 03 70 03

SECTION I

INAPPARENT CHOLERA INFECTION DURING THE NON-CHOLERA SEASON

William E. Woodward

SUMMARY

Blood specimens obtained from a subsample of the Matlab vaccine population in 1968-69 indicated that the bulk of inapparent infections occurred 2 months prior to the peak of the cholera epidemic. The hypothesis that a reservoir of inapparent infection must precede the symptomatic cases will be examined.

RESEARCH PLAN

A. Introduction and Specific Aims

The recognition of inapparent cholera infection comprising the bulk of infection during a cholera epidemic has occurred only recently. Van De Linde suggested in Hong Kong, during the epidemics of 1961-63, that there were perhaps as many as 100 inapparent infections for each apparent, i.e. severely ill, case (1). McCormack in 1966-67 demonstrated in Meharan the occurrence of significant inapparent infection during a period when there was not a single individual with clinical cholera requiring hospitalization. He found 5 people who were vibrio positive but who had only extremely mild diarrhea that would normally have been disregarded. In addition, there were 22 or more individuals whose sera demonstrated a four-fold or greater rise in vibriocidal antibody. Again in Meharan, during the 1968-69 cholera season, there was a total of 116 people who demonstrated bacteriologic and/or serologic evidence of infection. Of these, only 12 needed hospitalization.

During the cholera vaccination program in the Matlab vaccine trial surveillance area, in 1968-69, a 3% subsample of the vaccinated population submitted a fingertip blood specimen on 4 occasions. These were prior to vaccination in late August, 1968, just after vaccination in late September and early October, 1968, during the height of the epidemic in late December, 1968, and early January, 1969, and after the epidemic in mid-February, 1969. Serological analysis of these blood specimens for Inaba vibriocidal titer revealed 120 conversions, indicative of exposure to Vibrio cholerae, in those receiving placebo injections or no injections at all. Interestingly, approximately 75% of those conversions occurred in the period between the first and second bleedings. This was a time during which fewer than 4 isolations of Vibrio cholerae were made per week in the entire population of the area covered by the vaccination program. Later, during the peak of the epidemic when 20-45 isolations were being made per week, less than 25% of the conversions occurred.

These findings indicate the possibility, previously undescribed, that an epidemic of inapparent infection

may occur prior to an epidemic of overt cholera. In addition, a certain threshold level of inapparent infection in a population may indeed be required before clinical cholera becomes manifest to any significant degree.

To test this hypothesis and to confirm the above findings, it is proposed that a similar serological analysis be performed on a representative population in Matlab during the non-cholera season of the monsoon months and to compare these results with those obtained during the subsequent epidemic period.

B. Method of Procedure

Approximately 1,400 children under 10 years of age will be selected in a random fashion from 11 villages in Matlab. The villages selected will be compact and crowded and will have had a high incidence of cholera in the past. An attempt will be made to obtain a finger-tip blood specimen from each child in late June, early August, late September and at the peak of the winter epidemic. Subsequent serologic analysis for vibriocidal Inaba titer will be performed to determine at what point, if any, serological coversion occurs.

C. Significance

There is no precedent in the cholera literature for the peak incidence of inapparent infection to precede that of the symptomatic cases. It may be that, as is the case with meningococcal infection, a certain level of asymptomatic infections must be created before the disease becomes apparent. Such a situation would largely explain the seasonal nature of cholera in an endemic area, although it would not explain the seasonal character of the inapparent infection.

D. Facilities

All specimens will be obtained in Matlab by the staff presently employed and will be analyzed by the present Serology Unit staff. No new equipment or facilities will be required.

Reference

1. Van De Linde, P. M. A. and Forbes, G. I. Observations of the spread of cholera in Hong Kong, 1961-63. Bull. Wld. Hlth. Org. 32: 515-530, 1965.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
W.E. Woodward	Research Physician	30	-	-
K.M.A. Aziz	Sr. Investigator	10	40,320	4,032
6 (six)	Field Assistants	20	25,776	5,155
3 (three)	Dais	20	1,620	324
Mr. Huda	Field Surveillance Supervisor	5	15,936	797
1 (one)	Matlab Messenger	10	1,548	155
3 (three)	Country Boatman	10	3,600	360
1 (one)	Speedboat Driver	10	3,372	337
1 (one)	Statistical Clerk	5	8,352	418
Equipment:	None			
Supplies:	Medicines, micropipettes, lancets, cotton, methylated spirit, polyethelene bags, hard candy, balloons	$297		2,943
	IBM Cards			1,412
				35
Travel:	Dr. Woodward to EIS Conference in USA			7,600
Alterations and renovations:	None			
Publication Cost:				2,000
Other expenses	Secretarial costs:			500
	Food allowance for field personnel			2,400
	All serology costs (personnel and supplies)			2,200
	Titration	$187		890
	Preparation of vials			1,624
		$252		1,200
				34,457
	Overhead 30%			10,337
				44,794

PROTOCOL CODE 03 70 04

SECTION I

SEROLOGICAL STUDIES OF INAPPARENT CHOLERA INFECTIONS UTILIZING THE PF (TOXIN) NEUTRALIZING TEST OF CRAIG

Wiley H. Mosley
William E. Woodward

SUMMARY

A selected sample of the 3,000 sera collected as a part of the Meharan community study for inapparent infections, and of the 9,000 sera from the 4 serological surveys in the 1968-69 vaccine field trials will be titered for toxin neutralizing antibodies to define the serological response in inapparent infection, and to estimate the inapparent infection rate in the Matlab vaccine field trials.

RESEARCH PLAN

A. Introduction and Specific Aims

During the 1968-69 cholera season, an intensive epidemiological and serological study was carried out in village Meharan to determine the frequency of mild or inapparent infections with Vibrio cholerae. Three consecutive serological surveys were carried out on the entire population, rectal swabs were obtained from all individuals reporting diarrhea and rectal swabs were obtained from 100 children daily for the three-month cholera season. The bacteriological findings combined with the serological studies utilizing the vibriocidal test, indicated that 116 members of this community were infected with Vibrio cholerae, though only 12 required hospitalization. Examination of the sera from this population for toxin neutralizing antibodies will for the first time permit us to characterize the toxin neutralizing antibody response in mild or inapparent infection and also compare the sensitivity and specificity of the toxin neutralizing test with the vibriocidal test.

Approximately 2,000 children were bled on 4 consecutive occasions through the 1968-69 cholera season as a part of the vaccine field trials. Vibriocidal antibody titrations are being completed on these sera. This data will permit us to relate the cholera infection rate to the vibriocidal antibody response induced by the vaccine; however, the vibriocidal antibody test will be of no value in detecting inapparent infection in this population by virtue of the majority of them having received cholera vaccine. Previous studies in this Laboratory have demonstrated that the whole cell cholera vaccines do not induce toxin neutralizing antibodies. Therefore, determination of the toxin neutralizing antibody pattern in the various vaccine groups will permit us to estimate the inapparent infection rate due to Vibrio cholerae in these populations. From this data, it may be possible to actually estimate the vaccine effectiveness serologically based on the proportions of the population showing toxin neutralizing antibody conversions in the various vaccine groups.

B. Method of Procedure

Titrations will be carried out on all of the sera (300) obtained from the 100 children in Meharan who were studied most intensively bacteriologically. A selected sample of approximately 200 sera from the remaining Meharan village will also be studied. Based on these studies, it will be possible to determine the sensitivity and specificity of the toxin neutralizing test and compare the toxin neutralizing response with the vibriocidal antibody response.

Sera from approximately 500 persons selected to represent proportionately each of the vaccine groups will also be titered and a comparison of the responses in the vaccinated and control groups will be made.

The toxin neutralizing titrations will be carried out in the rabbit skin according to the standardized procedures established by Craig and the results will be reported in antitoxin units as defined by Craig, based on results obtained with a standard reference sera.

C. Significance of this Research

At the present time, there is extremely limited data on the toxin neutralizing antibody response, particularly during the course of inapparent infection. Preliminary studies carried out as a part of a family study two years ago suggest that approximately 50% of persons with inapparent infection may develop toxin neutralizing antibodies. If this finding is confirmed this, in itself, is significant since it indicates that a significant amount of toxin was produced in the intestine of the host to stimulate an antibody response even though diarrhea did not develop.

The definition of the toxin neutralizing antibody response in inapparent infection with cholera is essential if there is to be a proper interpretation of the naturally-occurring toxin neutralizing antibodies found in East Pakistani populations. From this study it may also be possible to further determine whether or not there exists any relationship between toxin neutralizing antibodies and resistance from cholera. The data available to date

suggests that such a correlation does not exist; however, the previous studies have been carried out utilizing a skin test which is sensitive to extremely low levels of toxin neutralizing antibodies.

D. Facilities

The facilities for this work are available. Because these are acute experiments involving rabbits, long-term maintenance facilities for rabbits are not required.

E. Supporting Data

The toxin neutralizing test was developed at this Laboratory by Craig in 1964. Since then we have had extensive experience with this test. Benenson published the toxin neutralizing antibody response during the course of cholera. Subsequently, we collaborated with Craig in a project relating the serum toxin neutralizing antibody titer to the ability to neutralize the skin test. This was followed by a study of the skin test response during the course of clinical cholera carried out by Eichner. Following this, Martin utilized the skin test to attempt to relate antitoxin immunity to resistance from cholera. The results were negative. More recently we have been able to demonstrate in this Laboratory that the skin test, measuring the neutralization of vascular permeability factor and the rabbit loop test, measuring the neutralization of the ileal loop toxin, are probably measuring the same antigen-antibody reaction, and that a single toxin is involved in both responses. This finding adds significance to any studies of skin toxin neutralizing antibody.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
W. H. Mosley	Division Head	10	-	-
W. E. Woodward	Research Physician	10	-	-
A. Mahmud	Research Veterinarian	40	15,312	6,125
	Lab. Technician	20	5,000	1,000
	Lab. Attendant	30	3,000	900
	Statistical Assistant	20	7,500	1,500
Equipment	None			
Supplies	200 Rabbits			15,000
	Tuberculin syringes			3,000
	Reagents			400
	Glassware			200
	IBM cards, stationery			300
Alterations and Renovations	None			
Publication costs				500
Secretarial				1,000
				29,925
Overhead 30%				8,978
Total				38,903

PROTOCOL CODE 03 70 05

SECTION I

1968-69 CHOLERA VACCINE FIELD TRIALS (COMPLETION OF ANALYSES)

Wiley H. Mosley
William E. Woodward

SUMMARY

Vibriocidal titrations will be completed 3,000 sera obtained during the 1968-69 vaccine field trials, and statistical analysis of the field trial results will be completed.

RESEARCH PLAN

A. Introduction and Specific Aims

The protocol for the 1968-69 vaccine field trial is described in detail in E/P17 in the 1968 Technical Committee Report. Basically, there were four vaccine groups: Vaccine F, a whole-cell Ogawa vaccine; Vaccine G, a purified Inaba antigen; Vaccine H, a whole cell Inaba vaccine; and Vaccine I, a tetanus and diphtheria toxoid control. These vaccines were administered to approximately 40,000 children in a double-blind, controlled study in September, 1963. A sample of approximately 1,000 children selected and were bled on four consecutive occasions, once prior to vaccination and three times following vaccination through the cholera epidemic. The specific objective of the field trial was to determine the degree of protection given by monovalent Ogawa and Inaba vaccines, as well as the protective efficacy of the purified Inaba antigen. The serological surveys were taken to define the vibriocidal antibody response following vaccination and to relate the vibriocidal antibody titer produced by the vaccines to protection.

This protocol covers the work that remains to be completed and the analysis of the results of the 1968-69 field trial. There remain approximately 3,000 sera requiring vibriocidal antibody titrations and, in addition, approximately 30,000 census records remain to be punched for computer processing.

B. Methods of Procedure

The vibriocidal antibody test will be carried out according to the standard microtitration techniques utilized in this Laboratory, and statistical analyses will be done as with previous vaccine field trials.

C. Significance of this Research

The case counts in the 1968-69 field trials indicate that both the whole-cell Inaba vaccine and the purified Inaba antigen produced excellent protection against Inaba cholera. The whole-cell Ogawa vaccine was essentially ineffective in children under the age of 5, although it had an efficacy of

about 50% in children ages 5-14. The results of the vibriocidal antibody titrations will be useful in interpreting the results of the field trials, in particular in attempting to characterize the nature of the cross protection produced by the whole-cell Ogawa vaccine against Inaba cholera. Previous field trials have suggested that the difference in protection in the older and younger children is due to the fact that older children have a high level of naturally-acquired immunity and that even a relatively weak vaccine acts as an effective booster. In this year's study it will be possible to determine whether or not the Ogawa vaccine produced different Inaba vibriocidal antibody responses in young and older children and whether this can be related to the differences in protection produced in these two age groups.

D. Facilities

Laboratory facilities are available for vibriocidal antibody titrations. The Statistical Branch has just received a new IBM punch, verifier and sorter which will greatly accelerate the statistical analysis.

E. Supporting Data

The results of previous vaccine field trials carried out by this Laboratory have been published in the literature.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
Wiley H. Mosley	Division Head	25	-	-
W. E. Woodward	Research Physician	10	-	-
	Lab. Technician	25	9,672	2,500
	Lab. Attendant	20	3,000	600
	Statistical Assistant	50	7,500	3,750
Equipment	Microtiter plates		$ 50.00	240
Supplies	Guinea pig complement		$2,250.00	11,000
	Media, etc.		$ 30.00	144
	IBM cards, stationery, etc.			1,200
Travel	Dr. Mosley to US-Japan Cholera Conference			7,500
Alterations and Renovations	None			
Publication Costs				1,000
Secretarial				2,000
				29,934
Overhead 30%				8,980
				38,914

PROTOCOL CODE 03 70 06

SECTION I

DEVELOPMENT OF AN *IN VITRO* ANTITOXIN ANTIBODY TITRATION TEST

Wiley H. Mosley
A. K. Bhattacharjee
Ansaruddin Ahmed

SUMMARY

Partially purified cholera toxin will be prepared according to the procedure described by Richardson. This toxin will be used as an antigen to develop an *in vitro* test, utilizing either a passive hemagglutination technique as described by Finkelstein, or a radial immunodiffusion technique as described by Richardson.

RESEARCH PLAN

A. Introduction and Specific Aims

Over the past four years, the immunology laboratory has utilized two *in vivo* neutralization tests to study toxin neutralizing antibodies in cholera. These are the permeability factor neutralizing test, described by Craig, and the ileal loop toxin neutralizing test described by Kasai and Burrows. These techniques have been utilized to describe the antibody response during the course of cholera and characterize the immunoglobulins responsible for neutralization. The vibriocidal test which is currently used in most epidemiological studies, suffers from a lack of specificity for infection with *Vibrio cholerae*. Vibriocidal antibodies are stimulated by infections with other organisms, including Brucella and Salmonella. Toxin neutralizing antibodies are more specific for infection with *Vibrio cholerae* whether or not the diarrheal symptoms are manifest. Toxin neutralizing antibodies are not stimulated by most of the presently-available cholera vaccines. The specificity of this test makes it an extremely valuable tool for epidemiological studies. At present, the requirement for a bioassay for toxin neutralizing antibodies severely limits the practical utilization of this test for field studies.

The primary requirement for an *in vitro* test for antitoxin antibodies is a relatively pure antigen. The feasibility of developing a simple *in vitro* test has been demonstrated by Finkelstein, who has utilized a highly purified toxin to sensitize chicken red cells for a passive hemagglutinating test. In testing 13 sera sent to him from our Laboratory he found an excellent correlation between the *in vivo* toxin neutralizing titers we had obtained and his results obtained by the *in vitro* test. More recently, Richardson has incorporated a purified toxin he has prepared into agar and measured antitoxin titers by the radial immunodiffusion technique.

The specific aim of this project is to prepare a partially purified toxin according to the procedures described by Richardson and utilize this antigen to develop an *in vitro* antitoxin titration test, either by

the passive hemagglutination technique or the radial immunodiffusion technique.

B. Method of Procedure

The toxin will be purified from crude culture filtrates according to the techniques described by Richardson (1). For the preliminary studies, the starting material will be toxin NIH Lot 001; however, more potent toxins prepared in our laboratories may be used once the techniques are standardized. At each step of the purified procedure, the product will be assayed for protein by the Lowry technique and for bluing doses (BD_7) and limit of bluing units (LB 1) according to the techniques described by Craig.

Since the requirement for a sensitizing antigen is not necessarily a high degree of purity, but only that the interfering antigens are below the level of detection, the material available after each step in the purification procedure will be utilized to see if it meets the requirement for specificity. Synthetization of the chicken red cells will be carried out according to standard techniques. The sensitivity and specificity of the test will be determined by testing sera with high vibriocidal antibody titers with and without antitoxin titers. The correlation of the in vitro test with previous results obtained by in vivo titrations of the same sera will be determined.

C. Significance of this Research

Because of the high degree of specificity of the toxin neutralizing test, a simple in vitro technique would greatly facilitate the epidemiological field studies. This test would be of particular value in determining the inapparent infection rate in the vaccine field trial populations where the cholera vaccine has already induced high levels of vibriocidal antibody titers. In addition, a simple in vitro antitoxin titration test is a base requirement in order to provide the necessary serological support for a toxoid vaccine field trial.

D. Facilities

The specific reagents and crude toxin necessary for

this work are available. In addition, equipment including a cold room, fraction collectors, Millipore filtration apparatus, and a high capacity refrigerated centrifuge are available.

E. Supporting Data

The personnel in the immunology laboratory have had broad experience with toxin neutralizing tests and standard techniques have been established for skin titrations as described by Craig and ileal loop titrations as described by Kasai and Burrows. In addition, we have established a modified technique for toxin-antitoxin titrations both in the skin and ileal loop, to increase the precision of the test. Dr. Bhattacharjee, who will be carrying out the purification procedures, has had considerable practical experience with essentially all of the techniques described in the protocol. Dr. Ahmed, who will be developing the immunological test, has had extensive experience in developing passive hemagglutination tests with vibrio antigens, as well as with all of the immunodiffusion techniques that may be utilized.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
W. H. Mosley	Division Head	20	-	-
A.K. Bhattacharjee	Res. Associate	50	21,216	11,000
A. Ahmed	Res. Associate	50	22,944	11,500
A. Mahmood	Res. Veterinarian	30	15,312	4,600
A. Majid	Sr. Res. Assistant	50	13,440	6,800
	Lab. Technician	50	5,000	2,500
	Lab. Attendant	30	3,000	900
Dr. Richardson	Consultant			6,100
Dr. Feeley	Consultant			6,100
Mr. Hochstein	Consultant			6,100
Equipment	Beckman D.U. Spectrophotometer			20,000
Supplies	Millipore filter pads, Diaflo membranes		$300	1,440
	Dextran sulfate, biogel		$100	480
	Reagents for buffers, media, etc.			2,000
	Tuberculin syringes		$ 70	3,380
	Glassware, columns			2,000
	200 rabbits			15,000
Travel	None			
Alterations	None			
Publication cost				500
Secretarial cost				1,000
				101,400
Overhead 30%				30,420
Total				131,820

PROTOCOL CODE 03 70 07

SECTION I

FURTHER STUDIES IN THE EPIDEMIOLOGY OF EL TOR CHOLERA

II. (A) Comparison of the spectrum of illness caused by serotypes of El Tor and classical Vibrio cholerae

(B) Definition of the viability and interaction of El Tor, classical and other agglutinable vibrios in nightsoil

Kenneth J. Bart

SUMMARY

Disease caused by El Tor Ogawa and classical Inaba Vibrio cholerae has been documented as having distinctly spectrum of illness and differential viability in nightsoil. This study will undertake to separate whether this difference is a function of the biotype or serotype of the organism by standard bacteriological and serological techniques of family study; and to define the epidemiologically suggested differences in the viability of El Tor Ogawa and classical Inaba in nightsoil.

RESEARCH PLAN

A. Introduction and Specific Aims

During a simultaneous epidemic of El Tor Ogawa and classical Inaba cholera in 1968-69 in Chittagong, significant differences were demonstrated in the spectrum of illness among family contacts of cholera patients and in the relative ability to isolate each organism from the environment. The infection to case ratio for El Tor Ogawa among family contacts was 38:1 as compared to 5:1 for classical Inaba. El Tor Ogawa was isolated ten times more frequently from the nightsoil than the classical Inaba. Whether these differences were a function of the biotype or the serotype or a combination of both could not be determined.

By standard bacteriologic and serologic family study techniques, 20 families of each of the two serotypes of El Tor and classical cholera will be studied in Dacca.

Viability studies of Vibrio cholerae have documented that the El Tor Ogawa persists as much as two and a half times longer than the classical inaba in water and food (Felsenfeld, 1963, 1965). There is, however, no data comparing the viability of El Tor and classical vibrios in stool. It is possible that the El Tor vibrio has a longer viability in nightsoil than the classical vibrio, which would help explain the observations made in Chittagong. On the other hand, the presence of mildly symptomatic and inapparent infections surrounding recognized cases of El Tor may play a role, by continuing contamination ofthe environment in the higher number of nightsoil isolations observed. The presence of biotype-specific bacteriophage (Kappa-phage), other agglutinable vibrios or inhibitor substances in nightsoil may also play a role in the inability to recover the vibrio from nightsoil.

This study will evaluate the relative viability of El Tor and classical vibrios and their interaction with AGs in nightsoil as well as search for the presence of Kappa phage and/or inhibitor substances in nightsoil.

B. Methods of Procedure

Twenty index cases of each of the two serotypes of the El Tor and classical Vibrio cholerae will be selected for study. The families of these patients will be visited as soon as possible after the patient's admission. Routine demographic data, rectal swabs daily for 10 days, and blood for vibriocidal titers on days 1 and 10 will be obtained. Water source and latrine sampling will be carried out daily for 10 days.

Strains of El Tor, classical and AG vibrios isolated from nightsoil, will be tested separately and simultaneously for their viability and interaction in nightsoil collected bimonthly from the Dacca Municipality Conservancy System. Kappa phage will be searched for in the nightsoil. A crude filtrate of the nightsoil will be made to define the presence of inhibitor substances.

C. Significance

The distribution of infection caused by the El Tor Ogawa vibrio has been documented to be milder and it is more widespread in its infectivity. The recognition that these differences are not only biotype specific but serotype specific will be of help in assessing the incidence and prevalence of cholera infection in the community.

The increased viability of the El Tor, the presence of Kappa-phage in nightsoil, specific inhibition of the classical vibrio by AG vibrios or the presence of specific classical vibrio inhibitor substances would help explain the documented differential ability to isolate the El Tor Ogawa and classical inaba vibrio from the nightsoil.

D. Facilities

Laboratory facilities are available for vibriocidal antibody titration and routine cholera bacteriology.

E. Supporting Data

The results of previous field investigations are now in press and in preparation.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
K. J. Bart	Research Physician	10	-	-
Dr. M. Khan	Epidemiologist	25	23,808	5,952
Mr. S. Islam	Field Assistant	25	18,192	4,548
Mr. Shahidullah	Sanitary Inspector	25	7,308	1,827
Mrs. D. Purification	Field Assistant	25	4,752	1,188
Mrs. Nahar	Field Assistant	25	4,752	1,188
Equipment	None			
Supplies	Swabs, media, etc. for 600 rectal and environmental cultures for a 10-day period			30,000
	Saline vials, serasharp and vibriocidal tests on 1,000 bloods			6,240
	Swabs, media, etc. 600 cultures for viability study			3,000
	Miscellaneous office equipment and supplies (mimeographing forms, pads, pencils, etc.)			1,000
Transportation	Daily 2 jeeps for field studies for 2-month period			2,580

Secretarial	1,000
Publication	2,000
	61,523
Overhead 30%	18,456
Total	79,979

PROTOCOL CODE 04 70 01

SECTION I

CHAMBER, CROSBY CAPSULE

Jon E. Rohde

SUMMARY

Using a specially designed chamber, small Crosby capsule biopsy specimens will be mounted allowing measurements of short circuit current and potential differences in normals and cholera patients. Simultaneous measurement of unidirectional fluxes will be performed using isotopes of sodium and chloride. Thus, ion movements across human tissue naturally affected by the toxin of Vibrio cholerae will be studied.

RESEARCH PLAN

The discovery by Ussing in 1951[1] that Anuran skin short circuit current (Isc) equals net sodium flux, led to a valuable new technique for quantifying fluid and electrolyte dynamics in many epithelia. Investigations of similarly mounted intestine and stripped gut mucosa have been performed for tortoise, rat, rabbit, frog, dog and human tissues. In many of these Isc was demonstrated isotopically to reflect movements of multiple ions, but in human ileum, the Isc/Na net flux ratio remained close to unity suggesting equality of these two parameters[2].

Recent studies using isolated rabbit ileal mucosa have demonstrated a slow but significant rise in Isc when the tissue was exposed to cholera culture filtrates, shown to be due largely to hyper secretion of chlorides[3]. Mucosa from cholera infected rabbit gut displays similar electrical properties. Although studies of transmural potential differences (P.D.) in cholera patients showed no significant change from convalescence[4], this does not rule out significant differences in Isc since P.D. is markedly effected by small changes in tissue conductance where Isc is not. However, human ileal mucosa exposed to cholera culture filtrate in a chamber demonstrated no change in Isc P.D. or net Na flux when compared to appropriate controls[2]. These human experiments are in striking contrast to the work Field and Greenough[3] in rabbit ileum and raise the important question of species difference in studies of the pathophysiology of cholera.

This protocol is designed to study the intestinal tissue samples from human patients enabling direct measurement of Isc, transmucosal P.D., and isotope fluxes in acute and convalescent cholera.

Methods

The biopsy sample chamber was built and tested in Copenhagen this May, using stripped rabbit ileal mucosa. Although the cross sectional area of tissue studied is only 7 mm^2, Isc per cm^2 equalled results obtained simultaneously in a 1 cm^2 chamber with tissue from the adjacent area of ileum. Furthermore, the published results (Schultz and

Curran[5]; Field and Greenough[3]) of Isc, PD., unidirectional Na fluxes, Cl^{36} fluxes, Q10, and effects of theophylline Quaban and DNP were all duplicated using this preparation technique. However, simultaneous label experiments were not attempted.

Rabbit Ileal Mucosa

In order to perfect techniques of handling tissue and operating equipment, six to ten trials using stripped rabbit ileal mucosa will be run. Gassing, thermostating, and measuring techniques will be refined and results compared with simultaneous runs on adjacent tissue mounted in a 2 cm^2 chamber as well as published data on the effects of temperature, glucose, theophylline and cholera toxin[3,5]. No isotope fluxes will be done at this stage.

Dog Biopsy Specimen

Using dogs from ongoing acute experiments biopsy specimens will be obtained to allow practise with both Crosby and Cooke capsules. The biopsy will be viewed under a dissecting microscope to check integrity of mucosa and dry runs to practise chamber mounting will be carried out. Four to eight experiments using fresh dogs will be then performed, obtaining biopsy, mounting and measuring electrical properties as in the rabbit experiments.

Normal Human Controls

Normal healthy adult volunteers, free of any GI symptoms, with Hct 33% and with stool exams will be hospitalized for three days. After history, P.E., Hct and stool exam for parasities and blood, a peroral biopsy capsule will be passed and located by X-ray in the upper jejunum. A single biopsy will be taken (day 2) and mounted in the chamber for electrical measurements. If stable readings can be obtained for 3-4 hours in the first 4-5 cases, subsequent biopsies will be exposed on mucosal side to NIH lot No. 1 after one hour, the response being followed for the duration of the viability of the tissue. Response to mucosal glucose will be used as the measure of viability of tissue. Patients will be discharged 24 hours after biopsy pending normal Hct and stool guaiac. Hopefully 12-16 normals will be studied in this fashion before the cholera season.

Cholera Patients

All adult patients admitted with clinical cholera to CRL wards will be candidates for this study. Following history physical exam, stool ME and Hct, all with complicating disease will be eliminated. This will include pneumonia, anemia with Hct 33%, ulcer history, blood or parasites in the stool, and evidence of malabsorption. A biopsy capsule will be swallowed and allowed to advance to the proximal jejunum as evidenced by X-ray. The specimen so obtained will be immersed in mammalian Ringer's gassed with 100% O2 and taken immediately to the Lab where mounting and electrical measurements will be done. After stable readings of P.D. and Isc are obtained for 30 minutes, both sides of the tissue will be bathed in 15 mM glucose. A repeat period of 15-20 minutes without glucose will precede exposure of the tissue to NIH lot No.1. After a steady current level is achieved a final stimulation with glucose will be performed. Because of the very thin connective tissue-free nature of the mucosal preparation, viability (at least for rabbit mucosa) extends for 4-6 hours. Thus, we will be free to investigate the effects of a number of substrates and drugs on the mucosa if the preparation works well. These might include glucose, glycine, cuabain, theophylline c-AMP, prostaglandin E etc. A repeat biopsy will be taken as soon as the patient has ceased stooling for one day. Patients will be observed in the ward for 24 hours following their last biopsy, discharge preceded by normal Hct and stool guaiac.

Unidirectional flux studies (To be attempted only if previous portion successfully completed)

Although it has been shown without exception that Isc in the frog skin equals the difference of tracer determined unidirectional Na (i.e. Net Na flux) the case in intestinal tissues is more complex. While Schultz has shown convincingly that in the rabbit ileum (muscularis and serosa intact) Isc and Na flux are equal[5], Field[6] (using stripped rabbit ileal mucosa) reports Isc as much as 2x Na net flux, the balance of the current supplied through anion secretion. If we find significant Isc differences between cholera and normal the changes in ionic movements responsible will be

investigated through simultaneous bidirectional labelling using first Na^{22} and Na^{24} and later Cl^{36} and Br^{82}. These techniques will require great proficiency in mounting the sample and handling isotopes and will possibly require a return to the animal models for further practise.

Significance of this work

The definition of the precise aberration (s) in salt and water movement which underline the diarrhea in cholera has been the object of many studies. The theory of Na pump inhibition was delineated through observation of Isc depression by Vibrio products in Frog skin[7]. Excessive permeability has been postulated through study of static rabbit ileal loops[8]. The diarrhea induced in cannine T-V loops by cholera culture filtrate has been described through tracer fluxes[9] and recently a whole new mechanistic view of cholera has emerged from the stripped rabbit ileal mucosa exposed to NIH No. 1 and other agents. In this study I propose to utilize mucosa from the naturally infected human cholera gut, comparing the Isc, P.D. and eventually isotope fluxes of a precisely known area of this tissue with a similar specimen taken in convalescence. The changes in Na and Cl movement as a result of infection with Vibrio cholerae in human intestine will be investigated.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
J. E. Rohde	Research Physician	80	-	-
Hospital	12 normals - 12 x 3 = 36 hospital days @ Rs 300/hospital day			10,800
	12 cholera patients - 12 x 6 hospital days @ Rs 300/hospital day			21,600
Reimbursement to patients. @ Rs 5				1,000
Equipment and Supplies	Chamber, electronics, circuits, motors storage batteries, support frame			960
	Micrometer			340
	Oxygen			240
	Biopsy Capsule (U.K.)			590
	Scintillation fluids and fluors			240
	Isotope (U.K.)			590
	Animals - 12 dogs @ Rs 60 10 rabbits @ 70			1,400
Labour	Maintenance			400
	Chemistry Laboratory			500
Travel	U.S. Meeting			9,000
				46,660
Overhead 30%				13,998
Total				60,658

References

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   Acta Physiol. Scand. 1951 23 110

2. Grady, G.F. et al Gastroenterology 1967 53 737

3. Field, M. et al - Preprint -"Cholera Filtrates Stimulate active anion secretion by Rabbit Ileum in Vitro" 1969.

4. Sachar, D.B., et al Gastroenterology 1969 56 512

5. Schultz, S.G. and Zalusky, R. J. Gen Physiol. 1963 47 567

6. Field et al Fed. Proc. 1969 28 651

7. Huber, G.S. and Phillips, R.A. Proceedings of the SEATO Conference on Cholera 1960 p. 37

8. Love, A.H.G. Gut 1969 10 105

9. Iber, F.L. et al Journal Clin. Inv. (abst) June 1968 p. 50a.

PROTOCOL CODE 04 70 02

SECTION I

EFFECT OF CHOLERA ON BODY COMPOSITION

M. M. Rahaman
A. Majid Molla
R. S. Hare

SUMMARY

Loss of plasma and extracellular fluid volume on admission will be estimated in cholera affected children on the basis of measurements carried out on admission and at convalescence. Plasma volume and bromide space will be measured by T-1824 and bromide dilution techniques respectively. Whole body density will be measured by under water weighing. During the summer months, a group of healthy children will be brought from a cholera affected area and their body composition will also be studied by the above methods.

RESEARCH PLAN

Although the majority of patients admitted at the PSCRL are children below 12 years of age and although there have been studies of stool electrolytes in children, so far no attempt has been made to study the source of the fluid lost. In adults, the source has been shown to be chiefly extracellular fluid. The study proposed here was started last year. Dehydration was studied retrospectively and the relationships among total body weight, bromide space and plasma volume in children were observed. This year, the study will be continued and in addition densitometry will be done on as many subjects as possible.

Materials and Methods

Children between ages of 5-12 years will be studied. During the cholera season they will be studied on admission and at convalescence. During the summer months healthy children from Narayanganj will be brought in for a day only.

As soon as a child with cholera is brought into the hospital his general condition will be noted. If time permits weight will be taken (nude) in the metabolic scale, and height measured in the stadiometer. A blood sample will be obtained on arrival from the femoral vein. (If the child is pulseless the weighing will be deferred till he has received some amount of fluid which will be measured.) The child will be treated routinely with fluids and furoxone.

At convalescence (approximately 24 hours after the last intravenous therapy) the child will be weighed again and the plasma volume and bromide space measured by T-1824 and bromide dilution respectively. Normal controls will be studied in the same way.

Whole body density will be measured in children who can be trained to perform under-water weighing. This measurement consists of weighing the child in air and again when completely submerged in water with simultaneous measurement of residual volume of air in the lungs. The whole body density values are used to calculate total body fat. From this value, fat-free body and total body water can be calculated.

Plasma protein concentration will be estimated from specific gravity values. Packed cell volume and haemoglobulin concentration will be measured by the usual techniques. All these measurements will be carried out on admission sample of blood as well as that obtained at convalescence or from the normal controls. Assessment of dehydration on admission is based on changes in concentration of plasma protein and hemoglobin as well as change in PCV between admission and recovery.

Significance

The study will provide information on the body composition of local children who form the majority of admissions into the PSCRL hospital. The retrospective study of dehydration will furnish data which may enable us to determine the source of fluid in cholera. Preliminary results have shown that about 80% of the weight loss can be accounted for by change in bromide space. If all of the loss can be accounted for by change in total body water, we can conclude that the extracellular fluid is the major source.

Supporting Data

Ten children with cholera have already been studied for their plasma volume on admission which was repeated at convalescence along with the measurement of bromide space, which gives an estimate of the extracellular space. It was found that the measured plasma volume on admission in a number of cases of severe dehydration was lower than that estimated retrospectively. Similar finding were also noted by Dr. Phillips' group at NAMRU II and Dr. Harvey's group at the PSCRL. Since the admission measurement seems invalid, the measurement of plasma volume should be deferred till the patient has recovered.

Facilities

Facilities for the measurement of plasma volume, bromide space and whole body density by under water weighing already exists in this laboratory; trained personnel are also available. Cholera affected children admitted into the hospital will be used as subjects for acute and convalescent studies. They need not stay in the hospital for more than 5-6 days.

Narayanganj is situated well within the epidemic areas affected by cholera almost every year. One of the investigators (Dr. Molla) comes from this area and initial contact has already been made to bring the healthy children into the hospital for a day only. Extra expenses involved will be their transportation and a meal from the hospital.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
M. M. Rahaman	Sr. Investigator	25	50,952	12,738
A. Majid Molla	Physician	10	19,332	1,933
R. S. Hare	Consultant	10	31,668	3,167
Gholam Rasul	Research Assistant	20	8,340	1,668
A. Wahed	Research Assistant	50	7,452	3,726
Ayub Bhuiya	Lab. Technician	50	3,672	1,836
Hospital 20 patients - 20 x 6 = 120 hospital days @ 300/ hospital day				36,000
Meals @ Rs 1.50 for 20 patients				30
Transportation to and from Narayanganj 20 trips @ Rs 12.28				245
Laboratory cost @ Rs 4.38/man hour for 100				438
Gifts for health volunteers @ Rs 10 for 20				200
Supplies	Syringes, Needles, Chemicals, T-1824			2,000
Equipment	None			
Publication				2,000
				65,981
Overhead 30%				19,794
Total				85,775

PROTOCOL CODE 04 70 03

SECTION I

HOSPITAL TRIAL OF AN ORAL SOLUTION OF GLUCOSE, GLYCINE AND ELECTROLYTES FOR MAINTENANCE THERAPY FOR CHOLERA

Richard A. Cash
David R. Nalin

SUMMARY

Data from balance studies suggest that an oral electrolytes glucose and glycine solution for cholera is more effective maintenance therapy than a solution of electrolytes and glucose alone. The initial series of studies suggest that the two-substrate solution may shorten duration of disease by 12-16 hours compared to the one substrate solution. This idea will be tested in a clinical trial.

RESEARCH PLAN

During the past year it has been demonstrated in careful balance studies that an oral glucose and electrolyte solution can reduce the intravenous fluid requirements of adult cholera patients by 80%[1]. A clinical trial of this method of therapy gave similar results at a field hospital during a cholera epidemic[2]. Use of oral solutions of glycine and electrolytes with and without glucose has also resulted in a marked reduction of intravenous fluid needs[3]. A further significant reduction in intravenous requirements is not feasible at this time because the average amount of intravenous fluid required to correct shock in cholera patients is relatively constant. The solution of glucose and glycine, however, might reduce gross stooling as well as maintain net positive fluid balance by promoting greater absorption. A reduction in gross stooling could reduce the patient's discomfort and duration of disease.

Materials and Methods

Physician, nurses, and para-medical personnel participating in the study will be trained in the use of oral therapy by one of the two senior investigators. Patients will be those ten years of age and older who are admitted to the San Lazaro hospital with a clinical diagnosis of cholera with no complicating illness. Rectal swab will be taken on admission and every twelve hours thereafter. The composition of the oral solution with glucose and glycine will be as follows (millimoles per liter):

Na	100	HCO_3	48
K	15	Glucose	110
Cl	67	Glycine	110

The control group will receive the same oral solution but without glycine, and with the glucose increased to 330 m Osm/liter to match osmolarity.

On admission alert patients who are not hypotensive will receive oral therapy alone. Patients who are hypotensive will receive intravenous fluid until they are hydrated. Patients requiring intravenous therapy will be assigned by random paris to the control or study group. Oral therapy will begin as soon as the patient is alert. Vital signs and intake and output records will be checked regularly and recorded every 4 hours. The solution will be given at a rate of 750 - 1500 ml./hour during the first 4 hours, depending on initial severity of dehydration and stooling rates. The volume of oral therapy given during any 4 hour period will equal the volume of stool and vomitus lost during the previous 4 hour period. If the volume of stool and vomitus during any 4 hour period exceeds oral intake, the difference will be replaced with intravenous fluid. Additional intravenous fluid therapy will also be given if hypotension or oliguria occurs.

Patients will not receive tetracycline. The oral solution will be warmed to 40-45°C. Diet will be allowed as tolerated when stool volume is under 600 ml. during a 4 hour period. Patients will be transferred to the convalescent ward when their stooling rate is 300 ml. or less during any 4 hour period.

Significance

A more effective solution could reduce gross stooling and stooling duration, possibly allowing ambulation of severe cases 12-16 hours earlier than at present. This would reduce nursing costs and free up scarce hospital beds.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
David R. Nalin	Research Physician	10	-	-
Richard A. Cash	Research Physician	10	-	-
Equipment				2000
Supplies	(Bact., Chem.)			8000
Hospitalization	100 patients at Matlab Bazar Hospital at 5 days/ patient at about Rs 100/day			50,000
Publication				1000
				61,000
Overhead 30%				18,300
Total				79,300

References

1. Nalin, D. R., Cash, R. A., Molla, M., Islam, R., and Phillips, R. A. The Lancet, August 17, 1968, p. 370.

2. Cash, R. A., Nalin, D. R., Reller, B., Rochat, R., Haque, Z., and Rahman, A.S.M. Manuscript in preparation.

3. Nalin, D. R., Cash, R. A. Manuscript in preparation

4. Nalin, D. R. and Cash, R. A. Manuscript in preparation.

PROTOCOL CODE 04 70 04

SECTION I

COLON IN CHOLERA

M. M. Rahaman
A. Majid Molla
Mrs. R.S.Hare

SUMMARY

The role of colon in altering the composition of diarrheal fluid from the small intestine will be studied in both adults and children. An electrolyte solution made up to resemble ileal fluid in cholera will be perfused through the colon and changes in its composition will be studied.

RESEARCH PLAN

It is comparatively easy to test the role of colon in altering the composition of cholera stool by infusing a "cholera-like solution" at the level of caecum. Levitan, Fordtran, Burrows and Inglefinger (1962) have used the same technique to study the absorption of water and sodium in healthy volunteers by infusing normal saline. A similar study is proposed, in which the colon of subjects of various ages will be perfused with fluid of the composition of diarrheal fluid from ileum. Studies will be made during active cholera as well as convalescence. The objects of the study are to compare colonic function at various ages and to compare cholera with recovery.

Materials and Methods

Subject: Males above the age of five who are not malnourished and/or suffering from any complication.

Methods: A double lumen tube will be used whose terminal openings would be situated 15 cm. apart from each other. The proximal opening, which should be located in the caecum will be used as infusion port, the distal opening will be used for sampling.

The perfusing solution will contain Na - 140, K - 10 and HCO_3 of 25 mEq per liter mixed with 50 mg./liter of BSP. The electrolyte concentrations approximate those of ileal aspirates from cholera patients. The solution will be warmed to body temperature before infusion and will be infused at 250 ml/hour for 6 hours. A preliminary infusion at 1500 ml/hour is required to wash out the bowel. A Foley catheter will be introduced per rectum for a distance of 10 cm. and will be inflated with 10-15 ml. of water. We have found that this facilities collection.

As soon as it is certain that the tube is in place i.e. past ileocaecal valve and lying in the caecum (to be ascertained by X-ray after injecting barium sulfate solution through the tube with proper shielding of chest and genitals) the patient will be taken to the study room. He will be weighed, and a blood and urine sample will be taken. He will not be given milk or solid foods for

8 hours prior to the study. Blood will be drawn at 0, 3 and 6 hours so as to follow the plasma specific gravity to detect haemodilution if it occurs. The study will be terminated if specific gravity of plasma becomes less than 1.022 at any time. Vital signs will be recorded at half hour intervals.

Samples will be collected from the 2nd port and from the Foley catheter as nearly continously as possible.

Urine passed will be collected and weighed and analysed for Na^+ and K^+. At the end of the study the child will be weighed returned to the ward on full diet. During the study, a watery solution containing 5% sugar will be allowed by mouth. A maximum of approximately 20 ml. of blood will be drawn from a patient weighing at least 12 kg.

Chemistry: Blood: Hct, plasma specific gravity and concentration of Na^+, Cl^- and HCO_3^-

Stool: Na^+, K^+, Cl^-, HCO_3^- and BSP

Urine: Na^+, K^+, Cl^-, HCO_3^- and pH

Significance

It has been suggested that a difference in colonic function exists between young children and adults, because the Na^+ concentration of the stool is lower in pediatric cholera. This study is an attempt to describe the effect of age as well as the effect of cholera on the colonic alteration of an "ileal" fluid.

Supporting Data

Both Drs. Molla and Rahaman have experience with pediatric patients and have intubated a number of children and adults. Some supporting data has already been collected in preliminary studies involving intubation and perfusion.

Eight children were intubated during active cholera where the tube was allowed to migrate up to the terminal ileum. Samples were collected every 20 cm. and simultaneous samples obtained from the rectum. Analysis showed that the average composition of fluid at the terminal

ileum was Na^+ = 138, K^+= 6, CO_2= 20 and Cl^-= 74 mEq/liter. Mahalanabis (personal communication) from the John Hopkins Center in Calcutta has studied a number of children in somewhat similar fashion. Although our results do not seem to agree with those reported by him, they tend to support the probability that major changes in the electrolyte composition of diarrheal fluid in cholera take place in the colon. A group of 14 children who were convalescing from cholera at PSCRL were also intubated with a single lumen tube and perfused from the caecum. The solution used was the same as has been proposed for this study. The results show that in eight out of 14 cases the Na^+ concentration in the 'stool' decreased from 140 to a level below 125 mEq/liter and the bicarbonate increased from 25 to nearly 40 mEq/liter. The greatest changes were in HCO_3 and Cl concentration; K was either unchanged or slightly elevated. These preliminary studies indicated that 250 ml./liter is an optimal rate of perfusion.

Facilities

The chemistry branch provides the analytical laboratory for this project. It has two flame photometers, three Van Slyke machines and facilities for the preparation of solutions which will be used for this study. The replacement of old flame photometer is the only major expenditure.

The hospital admits cholera patients of all age groups and there will be no problem in selecting the 30 patients required for the study during the epidemic. Nursing help will be available from the ward during the study.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
M. M. Rahaman	Senior Investigator	35	50,952	17,833
A. Majid Molla	Physician	20	19,332	3,866
R. S. Hare	Consultant	10	31,068	3,107
Gholam Rasul	Research Assistant	50	8,856	4,428
2 full time nurses for 60 hospital days (Mrs. Nessa and Mr.Khondakar)		20	16,416	3,283
2 sweepers from Administrative for 60 days		20	3,096	619
Laboratory Cost	@ Rs 4.38/man hour x 5000 hours			21,900
Equipment	Flame Photometer			12,000
Supplies	Chemical, syringes, needles			5,000
Hospital	30 patients 30 x 8 = 240 hospital days @ Rs 300/ hospital day			72,000
Travel				9,000
Publication costs				2,000
				155,036
Overhead 30%				46,511
Total				201,547

PROTOCOL CODE 04 70 05

SECTION I

OSMOLAR REGULATION OF INTESTINAL FLUIDS

Kendrick Hare
David R. Nalin
K. M. Ally

SUMMARY

If a hypertonic solution is introduced into the lumen of the small intestine, its osmolarity falls to the plasma level; if the solution is hypotonic its concentration increases. These changes occur regardless of the nature of the solute which may be either organic or electrolytic, non-absorbable or absorbed by active or passive processes. The mechanism by which this adjustment is regulated will be studied in the small intestine of the dog.

RESEARCH PLAN

A. Introduction and Specific Aims

Thirty years ago Visscher's laboratory varied the osmolar relations between plasma and fluid placed in the lumen of the dog small intestine. Water movement was correlated with sodium transfer (Am. J. Physiol 142 : 550, 1944). Recently Whalen (J. Clin. Invest 47:1071, 1968) showed that in hydrated normal man water and sodium were absorbed when isotonic salt solution was introduced into the jejunum. When exogenous vasopressin was given or when the endogenous hormone was released by dehydration of the subject absorption was abolished. We have therefore evidence that an osmotic gradient between plasma and luminal fluid modifies the transmural movement of water and that a hormone can also produce an effect even when no osmolar gradient exists.

We plan to extend the osmolar studies by using several solutes including one not absorbed and some absorbed by diffusion or by active transport. When this background knowledge is acquired we will repeat the series in dogs deprived of their internal source of vasopressin. The susceptibility of the regulatory process to cholera toxin will be defined.

B. Methods of procedure

The adjustment of the osmolarity of fluid in the small intestine of the dog will be observed in acute experiments in anesthetized dogs. The first observation will be on the upper jejunum with later studies on the duodenum and ileum. A perforated plastic tube will be passed throughout the loop for the introduction, mixing and sampling of the luminal fluid. Solutions of different concentrations of sodium, chloride, mannitol, urea or glucose will be placed in the loop.

The addition of a nonabsorbable marker will permit calculation of dilution or concentration by the loop. Cannulation of the venous return and a femoral artery will provide sera for measuring A-V differences. Total osmolarity sodium, potassium, bicarbonate, chloride, urea

and glucose will be determined on serial venous and arterial sera and on luminal fluid.

The course of the changes can be illustrated by plotting concentration of each solute against time. At least one study per hour can be carried out over three or four hour experiment.

When the pattern of response is established for the intact dog, the procedure will be repeated on dogs acutely hypophysectomized and on dogs with chronic diabetes insipidus following transection of the pituitary stalk. If the vasopressin deficient dog consistently differs from the normal in his intestinal response, replacement therapy with vasopressin will be tested.

The effect of cholera toxin will be studied by serial observations of the intestinal response to hyper - or hypotonic solutions before and after the intraluminal administration of an effective dose of NIH cholera filtrate.

We plan further to study the <u>in vitro</u> effect of varying the concentration of solutions introduced into isolated loops of the dogs intestine immersed in physiological solutions.

C. <u>Significance</u>

The functional defect in cholera has not been identified. The manifestation of this effect, the accumulation of a salt solution in the intestinal lumen, has been described repeatedly but the participation of hormonal and physical factors is almost unknown. This project is an exploration into the pathophysiology of cholera through an animal experimental model.

D. <u>Facilities</u>

The chemistry section provides the analytical laboratory for this project. It has two flame photometers, two osmometer, several spectro photometers, three Van Slyke machines and basic facilities for the preparation of solution to be introduced into the intestine.

The experimental room in the animal house is now equipped with an hydraulic operating table, instrument table, surgical lamps, water baths, a sink and cabinets for storage of supplies.

The facilities for conditioning pye dogs for experimental use are inadequate both in quantity and quality but are being improved.

The replacement of one old flame photometer is the only major expenditure.

E. Supporting Data

Each of us has some experience in the approaches to experimental cholera described above.

Dr. Hare has, over a period of several years, studied hormonal and physical factors in the regulation of the osmolarity of body fluids with especial emphasis on renal mechanisms.

Dr. Nalin has recently studied the response of the Thiry-Vella loop in dogs and sheep to cholera toxin, measuring the net flux of water and electrolytes.

Dr. Ally has had long experience in the circulatory derangements in human cholera beginning with her work with Dr. Rejane Harvey enquiring into the role of acidosis in modifying the vascular bed of the great thoracic vessels.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
W. K. Hare	Head, Pathophysiol.	20	46,235	9,247
David R. Nalin	Research Physician	40	-	-
Ruth S. Hare	Pharmacologist	10	31,668	3,167
Dr. (Mrs) K.Ally	Research Associate	40	17,712	7,084
Chemistry Laboratory		6010 Man Hrs		26,362
Equipment	Flame Photometer			12,000
Supplies	150 Dogs @ Rs 50			7,500
	Chemicals, drugs, plastics needles, syringes, pipets and other glassware			12,000
Travel	Foreign Dr. Nalin or Ally to Federation Meetings in U.S.A.			9,000
Alterations and Renovations	Extension of dog runs Receiving room in animal house			5,000
Publication Costs				2,000
All Other Expenses	Repair and Maintenance			2,000
	Secretarial			2,000
				97,360
Overhead 30%				29,208
Total				126,568

PROTOCOL CODE 04 70 06

SECTION I

THE INTERACTION OF TOXIN NEUTRALIZING AGENTS WITH CHOLERA TOXIN

Richard A. Cash
David R. Nalin

SUMMARY

The factor in cholera toxin responsible for the diarrhea can be completely removed by pretreatment with a number of agents. This inactivation, however, can only be accomplished if the cholera toxin is mixed with these agents prior to administration to the experimental animals. If the toxin is given first, the agents tested have had no effect. Finding a substance which would reverse the effect of the toxin would have important therapeutic and physiologic implications. This study is designed to test the effects of various agents on Thiry-Vella loops treated with cholera toxin prior to the introduction.

RESEARCH PLAN

A. Introduction and Specific Aims

Various adsorbing agents have been recommended and used in attempts to adsorb the vibrio or its free toxin in the cholera patient. During the past year and a half the effect of a number of these agents on cholera toxin has been studied in the dog Thiry-Vella loop model. Substances so far tested include the following: charcoal, kaolin, T-1824, specific immune serum, and dextran. None of these substances has been able to reverse the effect of cholera toxin if the toxin was introduced into the Thiry-Vella loop prior to the administration of the particular agent. It thus does not appear that adsorbing agents hold much promise in being able to reverse the effect of the toxin

Emphasis will now shift to those agents which have a more direct affect on the intestinal mucosal cell. Such an example would be CPC (cetylpyridinium), an agent which removes the brush border of the cell. In another example, work from Baltimore has suggested that stimulation of cyclic AMP is the cause of increased intestinal secretion. Thus an agent inhibiting cyclic AMP might also be tried. New agents to be tested in the loop will depend on recent advances in small intestinal or cholera physiology appearing in the world literature.

This study is a continuation of the work that has been underway for the past 1½ years.

B. Methods of Procedure

A 40 cm jejunal Thiry-Vella loop will be constructed in study dogs. One week after the operation a control response of the loop to NIH Lot 001 toxin will be determined. The dog will be anesthetized with pentobarbital and Foley catheters will be inserted into each stoma. The loop will be rinsed with 40 c.c. NS, followed by 90 c.c. of air. Twenty-five c.c. of NIH cholera toxin Lot 001 will then be introduced into the loop and kept there for 5 minutes at which time the loop will be again rinsed with 40 c.c. NS and 90 c.c. of air. All amounts recovered will be measured. The

fluid produced by the loop will then drain freely into a graduated cylinder and be measured hourly over an 8-hour period. Six days later the particular agent will be tested. The same procedure will be followed. After the second rinse with NS the test agent will be added (in a total volume of 25 c.c.) and kept in the loop for one hour. The clamps will be released at the end of the hour and hourly collections for the 8-hour study period will be made.

A control period will be repeated 6 days later if the agent has had any effect on loop output.

C. Significance

We are now able to shorten the course of diarrhea by eliminating the vibrios present and thus probably prevent further toxin from binding to the intestinal mucosa. The bound toxin, however, continues to have its effect. If an agent is found to "unbind" or reverse the effect of toxin, one would only have to rehydrate a patient and then give this agent; no further intravenous or oral therapy would be required. The character of such an agent might also point to just where the toxin might be having its effect.

D. Facilities

All the facilities are presently available. No chemical or bacteriological tests need be done.

E. Supporting Data

Both investigators are experienced in the operative procedure and the experimental methods employed.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
R. A. Cash	Research Physician	10	-	-
D. R. Nalin	Research Physician	10	-	-
S. A. Ansar	Animal Caretaker	50	4,920	2,460
	Animal Handler	50	2,880	1,440
Equipment	None			
Supplies	100 dogs @ Rs 50			5,000
	Cost/day after surgery @ Rs 25/dog			2,500
	Chemicals, drugs, syringes, glassware			2,000
Travel	To meeting in U.S. to present paper			6,500
Alterations and Renovations	None			
Publication Costs				500
All Other Expenses	Repair and Maintenance			500
	Secretarial			500
				21,400
Overhead 30%				6,420
Total				27,820

PROTOCOL CODE 04 70 07

SECTION I

ORAL MAINTENANCE OF PEDIATRIC CHOLERA PATIENTS WITH A SOLUTION OF ELECTROLYTES AND GLUCOSE

David R. Nalin
Richard A. Cash
Kenneth J. Bart

SUMMARY

Oral maintenance of adult cholera patients has made possible a 70-80% reduction in intravenous fluid needs. This study will apply the experiments in adults to pediatric cholera patients.

RESEARCH PLAN

Background

Oral lavage of adult cholera patients with a solution of electrolytes and glucose has been shown to reduce intravenous fluid requirements by 80%[1]. The electrolyte composition of the adult oral solution is related to the electrolyte composition of adult cholera stool. Recent studies[2,3,4] have defined the difference between the electrolyte composition of adult and pediatric cholera stool. The definition of the pediatric cholera stool composition makes it possible to design an oral therapeutic solution for pediatric cholera patients. Recent work indicates that this type of solution could also be used for adults[4].

Aim of this study

To study the effects of oral maintenance therapy with a solution of electrolytes and glucose in pediatric cholera patients.

Methods

Pediatric patients with a clinical diagnosis of uncomplicated cholera and a positive admission darkfield examination for Vibrio cholerae will be accepted for the study. The diagnosis of cholera will be confirmed from rectal swab cultures taken on admission and repeated daily.

Patients receiving oral solution will be rehydrated by the intravenous route for 4 hours with 5:4:1 containing 1% dextrose until vital signs are normal and plasma specific gravity is under 1.028. Initial stooling rates will be determined. Intravenous fluid will then be discontinued and fluid losses in stool and vomitus replaced with matching volumes of an oral solution of glucose and electrolytes. The oral solution will be administered via naso-gastric tube. The solution will be warmed to 40-45°C before administration[5]. Tetracycline 20-40 mg./kg. will be given orally on admission and via the tube every 6 hours thereafter. Nothing will be given by mouth except the oral solution and tetracycline. The rate of naso-gastric infusion will be compared to stool losses at hourly intervals, and oral intake during the each 4-hour period

will equal the previous 4-hours' volume of stool and vomitus.

Intake and output will be checked and recorded every 4 hours. Vital signs will be recorded every 15 minutes until stable, and hourly thereafter. Height will be measured on admission and weight on admission and every 4 hours. Specimens of blood and stool will be obtained for analysis on admission, after rehydration and at 8 hour intervals, or more often if indicated by balance records and vital signs. Aliquots of each voided urine will also be analyzed. Analyses of all specimens will include Na, K, Cl. Stool and blood CO_2 and plasma specific gravity will also be determined.

If plasma specific gravity rises above 1.028 after intravenous therapy is discontinued, additional intravenous fluid will be given until plasma specific gravity is under 1.028. Studies will be continued until no significant stooling occurs during 6 consecutive hours.

The electrolyte composition of the oral infusion solution will be:

Na	120	mEq/liter
K	25	"
Cl	97	"
HCO_3	48	"

Glucose (110/mM/liter will be added)

Significance

If oral maintenance of children with cholera is successful, the need for large amounts of intravenous fluid for treatment will be markedly reduced.

Facilities

All the necessary equipment is available at the PSGRL.

Supporting Data

Both of us have extensive experience in clinical

cholera (adult and pediatric) and in oral maintenance therapy.

SECTION II

DETAILED BUDGET

(NAMRU 2 - for information only)

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
Richard A. Cash	Research Physician	10	-	-
David R. Nalin	Research Physician	10	-	-
Kenneth J. Bart	Research Physician	10	-	-
Chemistry Technicians	1200 man hours @ Rs 4/hr.			4,800
Supplies				4,000
Equipment	None			
Hospitalization	15 patients for 7 days @ Rs 300/day/patient			31,500
Publication				1,000
				42,500
Overhead 30%				12,750
Total				55,250

PSCRL Cost	Travel to Manila - R. Cash	1,845

References

1. Nalin, Cash, Islam, Molla, and Phillips. Lancet, August 17, 1968, p. 371.

2. Griffith, G., Fresh, Watten, Villaroman. Lancet, June, 1967, p. 1197.

3. Rahaman, M. M., PSCRL. Unpublished data.

4. Cash, R. A. and Nalin, D. R. Unpublished data.

5. Love, A. H. G. J. Physiology, London, 185, 80P, 1966.

PROTOCOL CODE 04 70 08

SECTION I

THE INTERACTION BETWEEN MAN AND VIBRIO CHOLERAE

W. K. Hare
G. Curlin
K. M. S. Aziz
R. A. Cash
K. Ally

SUMMARY

The primary pathological process in cholera appears to be limited to the mucosa of the small bowel. Monitoring the small bowel contents, stool and blood may reveal the host and vibrio factors brought to play in the disease. We intend to investigate the microbial, immunological and chemical characteristics of the small bowel contents and stool in patients to describe the interplay among these factors during the course of cholera.

RESEARCH PLAN

A. Introduction and Specific Aims

Cholera is a self limited disease. Cholera patients continue to excrete vibrios, presumably still infective, for variable period of time after the clinical disease has terminated but during convalescence excretion of the vibrio stops. Infected family contacts with mild or no symptoms excrete vibrios for a brief period. The factors responsible for terminating diarrhea and eliminating the vibrio from the gastrointestinal tract are unknown. Also, the factors which permit the vibrio to colonize one patient and not another and to cause disease in some and not in others are yet unknown.

Cholera is essentially a small bowel disease so the events surrounding infection and termination of clinical disease would be best looked for initially in the small bowel of acute patients.

The contents of the small bowel and cholera stool of patients will be investigated, permitting the study of the interaction between the host and the vibrio. Once these host and vibrio factors are delineated in acute patients, persons at risk will be studied.

B. Method of Procedure

Patients to be studied will be nonpregnant adults with uncomplicated cholera who will receive routine intravenous therapy and no antibiotics. As soon as the patient is hydrated a multilumen radio-opaque polyvinyl chloride tube with mercury bag will be passed by mouth. After localization of the sampling port in the proximal jejunum, a luminal sample will be collected either by syphonage or with a fluid wash containing a nonabsorbable marker. Thirty minutes after the intestinal sample is taken, a stool specimen will be obtained via rectal catheter. Should the data from jejunal aspirates warrant further segmental studies, the tube will be allowed to pass to the ileum on some patients and the collection repeated through the remaining uncontaminated lumen in the tube. Following the last intraluminal collection the tube will be withdrawn.

The entire process will be repeated on the same patient on the mornings of the third, fifth and tenth hospital days, after overnight fasts. Blood samples will be drawn on day 1, 3, 5 and 10.

Each intestinal and stool specimen will be immediately divided into two portions. The first portion will be processed immediately for quantitative and qualitative bacteriological studies and determination of the dilution factor if irrigation was used. Bacteriological studies will include quantitative counts of Vibrio cholerae and isolation of colonies of Vibrio cholerae which will be examined for serotype and roughness. The second and larger portion will be refrigerated until centrifuged and filtered to remove bacteria. It will then be subdivided before freezing and stored in aliquots for the following studies.

The first aliquot will be assayed for phage content by dropping dilutions onto lawns of susceptible vibrios and observing lysis. Subsequent antibacterial factors may be searched for on strains of the vibrio resistant to the phage present in the stool or jejunal aspirate.

A second aliquot will be examined for antibacterial factors by simple serial dilution of samples added to inocula of Vibrio cholerae in broth culture. It is planned that, as work progresses toward purification of immunoglobulins in stool and in intestinal fluids, a more precise differentiation can be made between the action of antibacterial antibody and of nonspecific antibacterial elements known to be present in these fluids.

A third portion will be tested for toxin in rabbit skin and isolated rat epididymal lipocytes. The presence of free toxin inactivating substances will be assayed in still another sample by serial dilutions incubated with known amounts of standard toxin. The presence of immunologically intact but perhaps biologically inactive toxin will be determined by interaction with antitoxic antibody. This test will require the production of a precipitin reaction specific for toxin. Later, when purified globulin fractions are available, they will be investigated for antitoxin activity. Thus it will be possible to distinguish between specific antitoxic antibody and nonspecific toxin inactivating factors.

A fifth portion of the filtered material will be used as a bacterial growth medium.

It will be divided equally and one half boiled for 10 minutes to destroy antivibrio antibodies that might have been added by the intestine. Each half, boiled and untreated, will be divided again providing four culture media all of which will be inoculated with a standard Vibrio cholerae culture of a strain known to be a toxin producer. One boiled and one untreated sample will be incubated aerobically at 30°C; the other two anaerobically at 37°C. After centrifugation, the supernatants of all four will be assayed on rabbit skin.

Mucin and protein content of intestinal aspirates and cholera stool will be determined chemically.

C. Significance

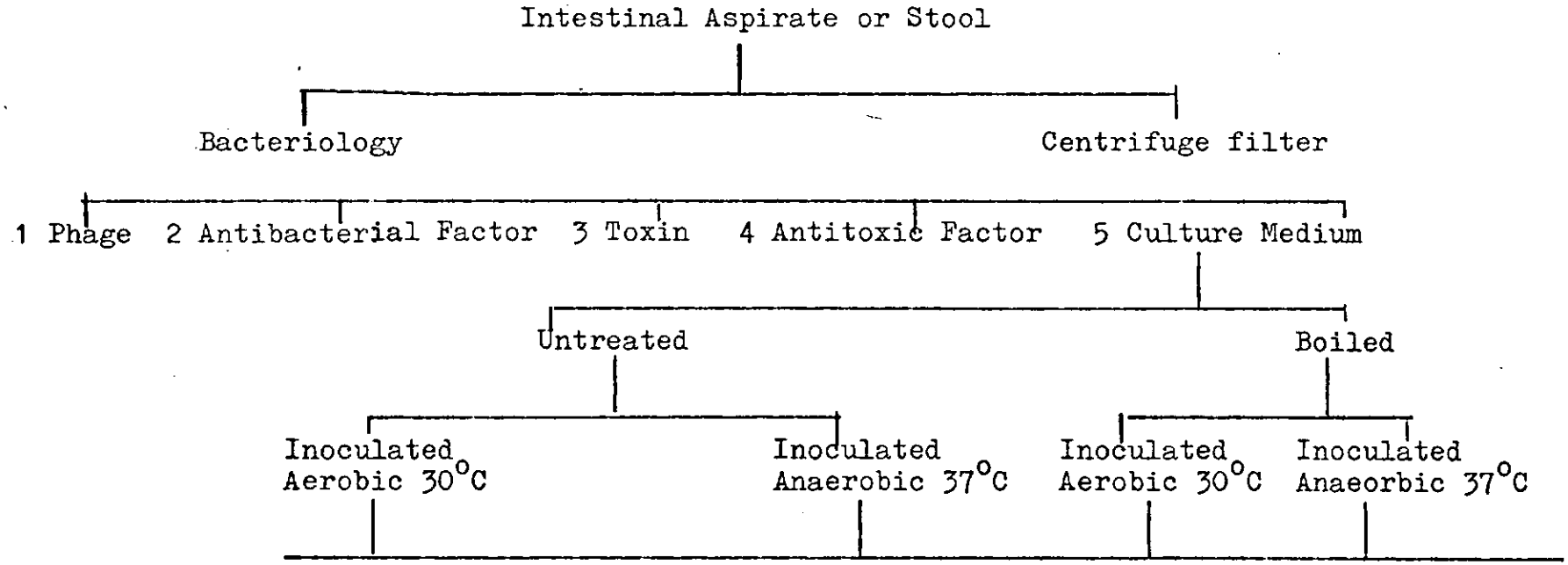
During the course of clinical cholera the number of vibrios present and amount of enterotoxin present in the succus or in the stool will provide data about the basic role of the vibrio in cholera and the changes that occur during the host response to the disease.

Similarly, the study of the immune response of the host during acute cholera, at the termination of the diarrhea and in convalescence, with emphasis on changes in antibacterial and antitoxic factors, both in the lumen and in the blood will aid the correlation of the systemic immunological response with the development of immunity. It is well established that the vibrio does not penetrate the mucosa of the bowel. Apparently large quantities of biologically active toxin do not gain access to the circulation of such, although small amounts of toxin or mediator substances may circulate. Conversely, recent work suggests that circulating immunoglobulins are not present in the intestinal lumen in quantities sufficient to protect against a large challenge. The mucosal plasma cell has been proposed as the mediator or the producer of intestinal immunity and the intestinal lumen as the site where the direct interaction between the host immune mechanism and the vibrio and toxin occurs. Therefore, a study of the small bowel and its contents during the course

of cholera should more directly describe how the immune response affects the course of cholera.

D. Facilities

This protocol utilizes many techniques that are familiar to the PSCRL staff. Among them are: the multilumen tube approach to the study of bowel function, identification and enumeration of Vibrio cholerae, bioassay of cholera toxin and antitoxin using the rabbit skin, methods of demonstrating antibody in intestinal contents and Ouchterlony double diffusion techniques. We will need to concentrate on developing other sensitive bioassays of cholera toxin which are within the capability of this Laboratory.



All to be assayed for toxin in rabbit skin.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
Dr. Kendrick Hare	Division Head	20	51,672	10,357
Dr. George Curlin	Research Physician	40	-	-
Dr. K.M. Ally	Research Associate	20	18,567	3,714
Dr. K.M.S. Aziz	Senior Investigator	20	52,994	10,985
Dr. Richard A. Cash	Research Physician	10	-	-
Mr. C. R. Dey	Supervisor	50	17,472	8,736
Mr. S.Z.A. Qader	Veterinarian	20	12,516	2,503
Mr. K. Alam	Research Technician	40	7,998	3,199
Mr. Abdur Rahim	Research Technician	40	6,120	2,448
Mr. Akter Hossain	Senior Research Asst.	40	14,592	5,837
Supplies	300 rabbits @ Rs 70			21,000
	50 dogs @ Rs 50			2,500
	Expendable Supplies: Culture media, Petridishes, syringes, filters			15,000
Hospital Costs	10 patients for 10 days @ Rs 300			30,000
Maintenance				10,000
Secretarial				2,000
				128,279
Overhead Cost 30%				38,484
Total				166,763

PROTOCOL CODE 04 70 09

SECTION I

RENAL FUNCTION STUDIES IN THE CHOLERA PATIENT

Richard A. Cash
Jon E. Rohde
David R. Nalin
Khairoon Ally
J. Satuffer Lehman, Jr.
Ruth S. Hare

Shock kidney with anuria is a recognized entity secondary to either hemorrhagic, traumatic, or endotoxic shock. Much of the early literature on cholera states that anuria is one of the major complications of the disease in both treated and untreated cases. There have, however, been no adequate reports in the literature evaluating renal function in the cholera patient. In the cholera patients brought to our hospitals within the first 24 hours of onset of disease (and often much later), and treated with the proper intravenous fluid, renal failure has rarely been observed. The purpose of this study is to define some of the basic renal function tests in the cholera patient and to study some of the effects of our present intravenous cholera therapy on this function.

RESEARCH PLAN

A. Introduction and Specific Aims

Anuria secondary to trauma, prolonged hemorrhagic or endotoxic shock is a well recognized entity. The cholera literature abounds with reports of anuria as a complication of cholera both treated as untreated. Though there have been few such reports since the introduction of modern intravenous cholera thereapy, this entity is still considered by many clinicians as a major complication of cholera. However, in a review of 320 adult (over 15 years of age) cholera cases treated at the PSCRL in Dacca between July 1, 1967 and July 1, 1969 there was only one case where anuria persisted for more than 20 hours. This patient had been anuric 24 hours prior to admission; good urine output returned after 48 hours in hospital.

There are no adequate studies reported in the cholera literature describing renal function in the cholera patient. There may be some mechanism by which the cholera patient protects his kidney from the severe prolonged hypotension, or the absence of anuria may reflect the type of therapy that the patient receives. For example, many patients brought to this hospital from other institutions, though adequately hydrated with normal saline, remained anuric until base was added to the intravenous fluid in order to overcome the acidosis that had never been corrected. Studies by Harvey et al in cholera patients have shown that severe vasoconstriction caused by the acidosis, is not fully corrected until the acidosis is corrected. Redistribution of blood flow under acidotic conditions may compromise renal circulation. The absence of K+ replacement in the N/S might also explain this anuria.

The purpose of this study, then, is to define renal function in the cholera patient using basic renal function tests. When these values are determined, we will look at the effects of different forms of therapy on renal function. It is important to note that this study makes no pretence at carrying out sophisticated renal function tests, but instead is an attempt to measure some of the basic parameters of renal function using uncomplicated well established tests.

B. Methods of Procedure

Male patients 15 years and older admitted with severe hypotension (systolic blood pressure <60) with clinical evidence of cholera will be candidates for the study. The following tests will be done on admission: blood (arterial) - Hct, plasma specific gravity, electrolytes (Na, K, Cl, HCO_3), creatinine, osmolarity, and pH. (Ca and Mg on admission, 24 hours and 5 days. A plasma aliquot will be kept of admission, 24 hours and 5 day blood.)

Patients will be hydrated with 5/4/1 and maintained on this intravenous preparation. Tetracycline, 250 mg every 6 hours will be given to all patients. Vital signs will be recorded every half hour until stable, then every 2 hours. Patients will be kept NPO during the first 24 hours except for oral water and tetracycline. Patients will have their bladders catheterized on admission; the catheter will be removed at 24 hours. The following studies will be done on the first urine specimen and at 2, 4, 6, 12, 18 and 24 hours. Analysis, creatinine, pH, Na, K, Cl, osmolarity. If the urine pH is >7 an HCO_3 will be done; if the urine pH is <7 titratable acidity and NH_3 levels will be carried out. Blood tests as above will also be repeated at the same times. All the above tests will be repeated once at 5 days.

C. Significance

This will be the first attempt to define renal function in the dehydrated and rehydrated cholera patient. It is essential to have a general knowledge of renal function in the cholera patient before more sophisticated tests are contemplated.

D. Facilities

The Laboratory is fully equipped to run all the tests listed.

E. Supporting Data

The first segment of the study is designed to define renal function in the cholera patient and as yet there is no useful information available on this subject. Once these baseline values have been determined, the direction of further studies will be decided.

SECTION II

Detailed budget for 12-month period from July 1, 1969 through June 30, 1970

Personnel	Position	Time	Annual Salary	Total
R. A. Cash	Research Physician	20	-	-
J. E. Rohde	Research Physician	20	-	-
D. R. Nalin	Research Physician	10	-	-
K. Ally	Research Associate	20	17,712	3,542
J. S. Lehman	Research Physician NAMRU-III, Cairo			
R. S. Hare	Experimental Pharmacologist	10	31,295	3,130
Chemistry technicians	375 man hours			1,500
Consultant: None				
Equipment and supplies:				5,000
Travel: To meeting in U.S. to present paper				6,500
Hospitalization: 75 patient days @ 300/day/patient				22,500
Outpatients: None				
Alterations and renovations:				1,000
Publication costs:				1,000
All other expenses				800
Sub-total				44,972
Overhead (30%)				13,492
Total				58,464

PROTOCOL CODE 04 70 10

STUDIES ON BODY COMPOSITION IN CHOLERA PATIENTS

M. M. Rahaman, R. S. Hare,
Majid Molla, and Waliur Rahman

Consultant - J. Rohde

Most of the cholera patients admitted to the Pakistan-SEATO Cholera Research Laboratory come from low socio-ecomonic classes. Although children below 12 years of age form the majority of patients admitted with cholera in this hospital, a large number of apparently otherwise healthy adults are also admitted. Most of the adult males lead an active life, as laborer or farmer with marginal existence incomes both of which contribute to the remarkable leanness of this population. As there is a marked difference between water content of fat and lean tissue it would be of interest to know which component contributes to the increase in weight seen beyond average height.

While studying the relationship between height and weight in cholera affected patients of Dacca (Music and Torrance, unpublished data) it was found that there was a close correlation between these two parameters up to a certain weight after which there was no increase in height with the increment in weight. (This is partly due to the short stature as well as the limited variability of height in average adult Bengali patients.) However, it would be interesting to know which component of body contributes to the increase in weight after a certain height is attained. It would be of interest also to observe the relationship between the body weight, height, total body water and the extracellular fluid volume which is the source of most, perhaps all of the diarrheal fluid in cholera. These informations would not only provide basic data on body composition in cholera affected population but will also help in elucidating the understanding of requirements of fluid in patients with varying body weight due to varying body composition. This will supplement the informations

already obtained on some 40 cholera affected children where blood volume was measured on admission and before discharge along with bromide space at convalescence.

Objectives

To study the body composition of adult subjects recently affected with cholera and of children if they can be trained for underwater weighing.

Materials and Methods

Subjects: The subjects will be adult and pediatric cholera patients who were discharged from the hospital during the recent epidemic. Attempts will be made to contact these patients and bring them to the hospital for one day.

Methods: Arrangements will be made to bring in the patients in the forenoon. Their weights will be taken on the metabolic scale with correction for clothes and then heights measured on the stadiometer.

Hydrostatic Weighing: While the patient will be waiting for equilibration of bromide to be established throughout the body his density will be measured by hydrostatic weighing.

Blood Volume, Bromide Space and Total Body Water: Blood will be collected for blank. 5 ml of T-1824 will be injected intravenously through the same needle or separately followed by an injection of 5% sodium bromide solution. The dose will be 2 ml/kg body weight. After 10 minutes a sample will be taken from opposite side of the body for the estimation of T-1824 concentration. After 3 hours another sample of blood will be collected for the estimation of bromide and tritium. Tritium dose is 1 µc/kg either i.v. or p.o. Volumes of distribution calculated by dilution of dose.

Harvard Medical School guideline for use of radiation tracers in man state that for normal volumes "tracer doses shall be limited to amounts such that the total absorbed dose does not exceed 2.5 rad. (0.25 rad. for children) in any twelve month period..." Our dose is less than 0.01 of that allowed per year.

Blood Samples: Admission: Hct, Hgb, Na, K, Cl, CO_2, and plasma protein. 10 minutes (opposite arm of injection) T-1824. 3 hours (opposite arm of injection) bromide , tritium.

Section II

Detailed Budget for First 12-month Period

Personnel	Description		Amount Requested	
Name	Title of Position	Time or effort % Hrs.	Annual Salary Rs.	Total Cost Rs.
M.M.Rahaman	Sr.Investigator	30	47,530	14,259
R.Hare	Consultant	10	31,068	3,107
A.Majid Molla	Physician	10	19,332	1,933
Waliur Rahman/ M.B.Roy	Physician	10	12,168	1,216
M.A.Wahed	Res.Assistant	50	7,896	3,948
Gholam Rasool	Res.Assistant	25	8,856	1,888
Ayub Bhuiyan	Lab.Technician	50	3,362	1,681
Laboratory Cost: Electrolytes, Plasma Sp.Gr.etc. 300 hours				1,328
Equipment: None				
Supplies: T-1824, Na-Bromide, Gold Chloride, O_2 cylinder etc.				2,500
Hospital: 50 patients 50 x 3 = 150 hospital days 150 x 300				45,000
				76,860
OVERHEAD 30%				23,058
			GRAND TOTAL	99,918

PROTOCOL CODE 05 70 01

SECTION I

STUDIES ON THE INHIBITOR(S) OF CHOLERA TOXIN OBTAINED FROM RATS

K. M. S. Aziz
R. A. Phillips

SUMMARY

Various substances are known to inhibit the action of cholera toxin. Potent inhibitor(s) aside from specific antibodies are also not known except a few substances that have been studied in our Laboratory. Studies will be carried out to find the nature and properties of some inhibitor(s) of cholera toxin, contained in rat serum and rat intestinal wash.

RESEARCH PLAN

A. Introduction and Specific Aims

During the last one year of toxin inhibition studies in our Laboratory "rat intestinal wash" (RIW) was found to inhibit the action of cholera toxin when mixed with the toxin prior to its introduction. In addition we have evidence that sera from rats and some other animal species can significantly inhibit the action of cholera toxin when used in this way. Inhibitors of cholera toxin e.g. kaolin, charcoal etc. are known (Finkelstein, Proc. Chol. Res. Symp. Honolulu: 264. U.S. Govt. Printing Press, 1965; Nalin and Cash, personal communication, 1969).

We plan to extend our studies on "rat intestinal wash" and rat serum. Emphasis will be put on the study of the nature and properties of the inhibitor in RIW and rat serum.

B. Materials and Methods

Conventional rat serum will be collected from our present rat colony. Dr. Feeley has provided us with a small amount of germ free rat serum. Request for more germ free rat serum will be made.

Goats will be immunized with NIH Lot 001 toxin and their sera collected and pooled. Thus a pool of 500 ml goat serum will be obtained. A pool of 250 ml of serum will be obtained from convalescent cholera patients. These two pools of sera will serve as controls for the experiments with rat serum and RIW.

Starting with a 100 c.c. of RIW it will be determined whether there is spontaneous breakdown of the toxin inhibitor. If there is no significant breakdown of the toxin inhibitor then a ten liter pool of centrifuged and millipore sterilized RIW will be made. This RIW pool will be concentrated ten-fold by negative pressure ultrafiltration.

Rat feces, urine and liver samples would be collected from our present rat colony. Rat food for test would be randomly taken out of the feed on the day of the experiment.

NIH Lot 001 toxin and Craig's reference serum will be used as standards for establishing the potency of the various sera and RIW. There is a skin permeability factor in rat serum. The relationship between the permeability factor and the permeability factor inhibitor in rat serum is a complication of skin tests and makes an extra set of control sera necessary.

A colony of Sprague - Dawley rats will be started in the animal house of our laboratory for collecting and testing toxin inhibitors produced by this strain of rats.

The first titrations will be carried out by the Craig's skin test procedure. After their potency has been measured in this way the inhibitors in the serum and RIW will also be titrated in adult rabbit small intestine loops essentially following the technique of Kasai and Burrows (J. Inf. Dis. 116: 606, 1966). The potency of the inhibitor present in the rat feces will be determined. Urine and liver samples and rat food will be tested to determine whether they contain toxin inhibitor. If toxin inhibitor is present in urine, liver or food of rats, the potency of the inhibitor in these samples will be determined.

Vibriocidal action of rat serum and RIW along with the two control sera will be checked.

Reversal of toxin action will be investigated in the following way. Adult rabbits will be operated and five ileal loops will be made. In all these loops 2 ml of NIH Lot 001 toxin in a total volume of 5 ml will be injected. 2 minutes or 10 minutes later the fluid inside the loops will be drained off and the loops will be rinsed with isotonic saline. The first loop will be untreated after the rinse. The rest of the loops will receive rat, goat and human sera and RIW. 4 rabbits will be used in each test so that each sample can be introduced into each loop.

Preliminary studies showed that RIW and rat serum was heat stable. This will be further investigated by heating at 56°C for 30 minutes and by boiling. Other attempts to inactivate the inhibitor will be: treatment with 2-mercapto-ethanol as well as strong acid or base, and finally with proteolytic or other enzymes. Goat and human immune sera

will be treated in the same way and used as controls in test animals. All assays for this purpose will be carried out in the adult rabbit skin.

Other methods of characterizing the inhibitor will be: ultrafiltration, molecular sieve filtration and density gradient centrifugation. Attempts at purifying the material will be made.

Finally, the inhibitor will be tested for antigenicity. Rabbits will be immunized with the partially purified rat serum and and RIW and the immune sera will be used for neutralizing the toxin inhibiting factor of rat serum and RIW in rabbit skin. Immunoelectrophoresis will be carried out on the immune sera thus obtained.

C. Significance

Potent toxin inhibitors other than specific antibodies and rat serum are not known. This project will try to find out the nature and properties of rat serum and RIW.

D. Facilities

The chemicals, glassware, surgical instruments and other facilities are already available in the laboratory. The animal operation room in the animal house is adequate and has operating tables, surgical lamps, a sink and cabinets for storage of supplies.

The only major expenditure is the cost of procurement of rabbits.

E. Supporting Data

We have reported that RIW inactivates cholera toxin (Aziz, et al, Nature 220: 814, 1968). We also have evidence for the heat stability (56°C for 30 minutes) and non-dialysability of RIW. Studies in our laboratory indicate that rat serum inhibiting factor is stable at 99.5°C ± .5°C for 30 minutes. Preliminary data show that .5 ml - 1 ml of conventional rat serum (from our colony) neutralizes 1 ml of NIH Lot 001 toxin. Germ free rat serum of Fisher

strain has about the same potency as the conventional rat serum. However, our data indicate that Sprague Dawley germ free rat serum is about ten times as potent as conventional rat serum tested.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
R. A. Phillips	Director	5	71,184	3,559
K. M. S. Aziz	Senior Investigator	40	52,944	21,178
A. K. M. Mohsin	Research Associate	40	19,332	7,733
S. Z. A. Quader	Veterinarian	40	10,764	4,306
G. K. Bhuiyan	Research Assistant	40	8,388	3,355
K. Alam	Research Technician	40	7,920	3,168
A. Rahim	Lab. Technician	40	5,964	2,386
J. Rashid	Lab. Technician	40	5,664	2,266
A. Jalil	Lab. Attendant	50	3,096	1,548
F. Mian	Lab. Attendant	50	2,232	1,116
Supplies	300 Rabbits @ Rs 70 per Rabbit			21,000
	2,400 Rats @ Rs 6 per Rat			14,400
	Chemicals, Drugs, Plastics, Needles, Syringes, Laboratory Glassware etc.			12,000
Travel	Foreign: To meetings in U.S.A.			9,000
Publication Costs				2,000

All Other Expenses	Repair and Maintenance	1,000
	Secretarial	600
		110,615
Overhead 30%		33,185
Total		143,800

PROTOCOL CODE 05 70 02

SECTION I

BLOOD GROUPS, SECRETOR STATUS AND CHOLERA

Kenneth J. Bart
Clarissa R. Bart

The relationship of genetics to pathological states is well established. Correlation between blood groups and disease and the possible effect of secretor status on disease have been reported. These associations have been most specifically related to diseases of the gastrointestinal tract. The correlation between blood groups, secretor status and a propensity to cholera will be studied.

RESEARCH PLAN

A. Introduction and Specific Aims

The relationship of genetics to pathological states is well established. Associations between blood groups and noninfectious diseases have been reported relating the ABO blood group system to increased susceptibility to venous thromboembolic disease[1], duodenal and gastric ulcers[2], carcinoma of the stomach, colon and rectum, pernicious anemia and diabetes mellitus[3]. There has also been suggestive evidence correlating blood groups to susceptibility to infectious diseases by relating the similarity, and therefore tolerance and susceptibility by the host, of micro-organism antigens to those of ABO blood group[4]. In other cases susceptibility to infection has been correlated to the induction of enzymes by gut bacteria specific for the breakdown of ABO blood group substances[5]. Clinical and epidemiological studies have demonstrated that the morbidity and mortality in smallpox was greater in group A and AB persons than other groups[6]. There is, however, some conflicting data and the issue remains unresolved[7].

The ABO blood group antigens are found in secretions throughout the body and on the surface of epithelial and endothelial cells. Not all individuals secrete the blood group substances. In the U.S. about 75% of white persons secrete the glycoproteins of the ABO blood group substances into their secretions[8]. In West Bengal, however, only 50% of persons were found to be secretors[9]. Persons of group O and nonsecretors of all ABO groups have a higher frequency of duodenal ulcers presumably related to the fact that ABO blood group substances are not secreted into the gut "to provide or associate with protection"[2].

Between December 1963 and May 1966 the ABO blood groups of 3,024 patients admitted to PSCRL were determined. A review of this data demonstrates a significantly higher frequency of group O and a lesser frequency of group B among cholera patients as compared to a normal blood group distribution of persons in West Bengal (Figure I).

It is likely that many factors, genetic and environmental contribute to the pathogenesis of cholera. If the propensity to cholera is in fact enhanced by genetic factors, evidence to support this may be found by further blood grouping studies and the determination of secretor status in cholera and non-cholera diarrhea patients. It is hoped that these associations will lead to an understanding of some of these factors.

This study will look at the correlation between cholera patients and their blood group antigens and their ability to secrete blood group substances by routine blood grouping and salivary secretor status methods.

B. Methods of Procedure

Blood samples will be collected from cholera patients admitted to the PSCRL and Matlab wards. Healthy, non-hospitalized, unvaccinated persons from the Matlab VTS without a history of cholera requiring hospitalization with and without significant vibriocidal titers will be used as controls.

4 ml. of blood will be drawn into 1 ml. of ACD (acid-citrate-dextrose) solution and the samples will be refrigerated for up to 21 days, until a workable number of specimens have been collected. The cells will then be tested with anti-A and anti-B typing serum. The plasma of each patient will also be tested with known cells of group A and group B to establish the presence of isoagglutinins. The cells of the patients will also be tested with anti-D (Rho), anti-E (rh"), anti-c, (hr'), anti-e (hr"), anti-M, N, S, s, anti-Kell (K), Cellano (k), Duffy a and Duffy b (Fy).

Blood group secretor status will be determined on saliva from cholera and non-cholera patients by the hemagglutination inhibition technique using anti-H substance derived from Ulex europaeus. The saliva of group A, AB and B non-secretors will be double checked with anti-A or anti-B.

C. Significance

This project is an attempt to identify a distinct blood group and secretor population with a propensity to cholera.

D. Supporting Data

Mrs. Bart has the technical experience for carrying out the appropriate immunologic assays.

E. Facilities

The pathophysiology clinical pathology section offers enough refrigerator space to store samples and a working supply of typing serums. An existing dry incubator can be adapted. A lab bench and chair will be required in the office shared by Dr. Nalin, Dr. Cash and Mrs. Bart.

The purchase of a serofuge and viewing lamp will be the major equipment expenditures.

BLOOD GROUPS IN CHOLERA

PSCRL ADMISSIONS BETWEEN DECEMBER 1963 AND MAY 1966

	Cholera			Non-Cholera		
Blood Group	Number	%	p*	Number	%	p
O	453	38.06	0.006	576	31.40	
A	280	23.52		527	28.73	0.061
B	340	28.57	0.002	574	31.29	0.041
AB	117	9.83		157	8.56	
Total	1190			1834		

DISTRIBUTION OF BLOOD GROUPS IN WEST BENGAL (CHAUDHURI, 1969)

Blood Group	Number	%
O	186	31.40
A	147	24.78
B	212	35.75
AB	48	8.09
Total	593	

*As calculated by the "t test" comparing the distribution of blood groups in cholera cases to the distribution in West Bengal (Chaudhuri, et al. Am. J. Phys. Anthrop. 30, 129-132, 1969).

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Total
K. J. Bart	Research Physician	5	-	-
C. R. Bart	Research Associate (part time)	50	8,748	4,380
Equipment	Sero-fuge complete with base, 12 place head and 12 sero-tubes. Clay Adams, Ind. Parsippany, New Jersey 07054. Sero-fuge (CT 1600) Base(CT-1605) 12 place head (CT 1610). Sero-tubes (CT 2130) Wt. 21½ lbs. shipping wt: 241 lbs.)			510
	Test tube agglutination viewer:(with 25 watt bulb and magnification mirror. Clay Adms, Inc. Viewer, complete (A 2330)			135
	Replacement bulb for above (6). Clay Adams, Inc. (A 1493). $1 each.			30
	Sero-tubes 3" x 3/8" (10 x 75 mm) $8.10 per gross. 2 gross.			81
Supplies	Vacutainers, formula 24, 1 ml. ACD solution B (2100). Becton Dickinson and Co., Rutherford, New Jersey07070 (S 3204X)			2,655
	Needles, 20G 1½" $2/doz. (3200 N)			1,750
	Plastic holders - 1 doz. (3200 H)			10
	Typing serum: Spectra Biologicals, Los Angeles, California. 10 ml. vials of each (10) Anti-A, B, Al, D (Rho), C (rh'), E (rh"), c (hr') e (hr"), M, N, S, s, K (Kell), k (Cellano) Fy^a (Duffy), Fy^b (Duffy, H (Ulex europaeus), Human gloculin (Coombs). $ 750.00			3,750

Miscellaneous	Saline, cotton, saliva containers, rubberbands, etc.	500
Publication cost		1,000
Secretarial		2,000
		13,321
Overhead 30%		3,996
Total		17,317

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2. Clarke, C. A., et al. Secretion of Blood Group Antigens and Peptic Ulcer. Brit. Med. J. 2: 43, 1958.

3. Race, R. R., and Sanger, R. Blood Groups in Man. Fourth edition. Oxford: Blackwell Scientific Publications. 1962.

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5. Hoskins, L. C. Ecological studies of Intestinal Bacteria. Relation Between the Specificity of Fecal ABO Group Antigen-degrading Enzymes from Enteric Bacteria and the ABO Group of the Human Host. J. Clin. Invest. 48: 664-673, 1969.

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9. Chaudhuri, S. B., et al. Study of Blood Groups, ABH Secretors and Hemoglobin Varients in the Upper Castes of West Bengal, India. Am. J. Phys. Anthrop. 30: 129-132, 1969.

PROTOCOL CODE 05 70 03

SECTION I

IMMUNITY TO CHOLERA IN THE UNDER ONE AGE GROUP

Kenneth J. Bart
George T. Curlin

SUMMARY

The under one year age group is spared from cholera relative to other age groups. Vibriocidal antibody is present in cord blood. Colostrum in diseases such as polio have been demonstrated to have clear protection in preventing intraintestinal infection with the wild or vaccine strains of polio virus. The role that transplacental antibodies and colostrum play in providing persisting cholera immunity during the first year of life is unknown. This study will investigate the role of transplacental immunity, colostrum and mothers' milk in the sparing effect noted in the under one age group.

RESEARCH PLAN

A. Introduction and Specific Aims

Analysis of the age distribution in the PSCRL hospital and experience with bacteriologically-confirmed cholera cases and acute diarrhea of undetermined etiology in an endemic cholera area revealed very few cholera cases under the age of one year, with cases increasing in frequency to the age of 4. Acute diarrheas, in contrast, had the highest incidence in children under the age of one year, with a progressive fall in cases with age. A similar pattern of sparing in the under one age group has been demonstrated both in Manila and Taiwan nonendemic areas. However, some inapparent infection has been demonstrated in this age group. In Meharan, Woodward demonstrated that the under one age group representing 2.9% (36 of 1,231) of the population contributed only 0.9% (1 of 116) of the total number of infections (1 inapparent case), whereas the 1-4 age group, representing 17% of the population contributed 50% of the total number of the infections in the community. Only 2.7% (1 case) of the under one age group were infected, whereas 25% (58 of 208) of the 1-4 age group (7 hospitalized and 51 infections) were infected.

In the PSCRL hospital of the nearly 5,000 cholera admissions there were only 25 cases (0.5%) of the cholera under one year of age. In Matlab of the nearly 3000 cholera admissions there have been only 5 cases of cholera under one year of age. Analysis of the age distribution showed the incidence of male and female cases up until 7 months of age was equal; however, at 7 months there is a rise in male cases.

The role of transplacental antibodies in the protection of the newborn has been well-documented for bacterial diseases, e.g. tetanus and diphtheria, and viruses, e.g. rubella, rubeola, chicken pox, polio, mumps, hepatitis and herpes simplex. Mosley, et al have shown that vibriocidal antibody is present in cord blood and is in the 7s fraction. Although the 7s fraction is that fraction which represents the response to acute infection in the adult the presence of this fraction in the newborn has

not been followed prospectively to measure its persistence or its role in protection when the infant is challenged.

Colostrum has been demonstrated to contain bacterial antibodies, e.g. diphtheria, tetanus, typhoid and H. pertussis, and viral antibodies, e.g. influenza, polio, Japanese B encephalitis and vaccinia. Nordbring demonstrated that antibodies against enteric pathogens appear in human colostrum in high concentrations. In general the antibody content decreases within a few days but persists in low titers in mothers' milk. Whether the infant absorbs antibodies is unclear. Neutralizing antibodies against polimyelitis have been demonstrated in colostrum and milk, but absorption could not be shown. Colostrum with such antibodies have been shown to prevent intraintestinal infection with the wild or vaccine strains of polio virus.

The character and persistence of cholera antibodies passed transplacentally in colostrum and in milk will be studied.

B. Method of Procedure

In Meharan, a village of approximately 1,700 people, 80 births are expected over the next year. A newborn clinic will be established and newborns will be followed prospectively. Prenatal blood, day 5 and monthly blood will be obtained from each mother. Cord blood, day 5 and monthly bloods will be obtained from each infant. Colostrum will be obtained daily for 5 days and milk monthly for up to 11 months or until the mother stops breast feeding; whichever is sooner. Blood, colostrum and milk will be analyzed for vibriocidal, agglutinating and toxin neutralizing ability. Some sera will be fractionated to determine the character of the antibody by sucrose gradient. Cohorts will be followed through two cholera seasons.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
K. Bart	Research Physician	60	-	-
G. Curlin	Research Physician	20	-	-
B. Sau	Nurse	30	12,000	3,600
	Field Assistant - Male	100	3,432	3,432
	Field Assistant-Female	100	1,800	1,800
	Laboratory Technician	25	5,000	1,250
	Laboratory Technician	25	5,000	1,250
	Laboratory Attendant	25	3,000	750
Equipment	Examining table, desks, etc. may be obtained from excess furniture at Matlab. Breast pumps (20) charis, benches.			500
Supplies	Neonatal kit, saline/vials, lancets, capillary micropipettes, alcohol, cotton, syringes, needles, miscellaneous medicines, candy, mimeographing, stationary supplies, balloons, IBM cards, towels, soap, etc.			4,300
	Hyland IgA, IgM, IgG antisera already in stock. DT vaccine donated already by Eli Lilly.			
Alterations	Renovation of house supplies by Meharan village			1,000
Publication				500
Secretarial				1,000
Other expenses	All serology costs (toxins, agglutinating, vibriocidal)			10,000
				29,382
Overhead 30%				8,814
Total				38,196

PROTOCOL CODE 05 70 04

SECTION I

INHIBITION OF CHOLERA TOXIN BY AROMATIC POLYSULPHONIC ACIDS

K. M. S. Aziz
R. A. Phillips

SUMMARY

Aromatic polysulphonic acids (APA) of known molecular structure can inhibit the action of staphylococcal toxin. Work in our Laboratory indicated that several aromatic polysulphonic acids also inhibit the action of cholera toxin. Using animal models, the effect of APA on the action of cholera toxin in the intestine, before and after the intestine has been exposed to the toxin, can be determined. The active site of cholera toxin molecule may be blocked by several APA.

RESEARCH PLAN

A. Introduction and Specific Aims

Studies with aromatic polysulphonic acids during the past few months have revealed that some APAs significantly inhibit the actions of cholera toxin when premixed. These studies were carried out mostly in the skin of adult rabbits. Arbuthnott and his co-workers (Biochem. J., 108: 41-48, 49-55, 1968) have demonstrated inhibition of staphylococcal α-toxin by several APAs. They introduced the APAs before and after exposing mice to staphylococcal toxin. One APA resulted in complete inhibition when injected 15 minutes before or after injection of the α-toxin.

We plan to study the effect of various APAs at different molar doses and a fixed dose of toxin at different pH. We also plan to study in detail those APAs which are significant inhibitors of cholera toxin when premixed. To test their ability to inactivate absorbed toxin these APAs will be introduced into animal models which have been exposed to the cholera toxin. Finally the toxicity of the APAs will be investigated.

B. Materials and Methods

Twenty-one aromatic polysulphonic acids and one aromatic sulphonic acid will be used at various doses.

Toxin NIH Lot 1 will be used for the various tests. NIH Lot 1 toxin is distributed by NIH, Bethesda, Maryland, in bottles containing 100 gms. of lyophilized crude toxin. To reconstitute this toxin to its original strength 100 gms. is diluted to 4000 ml. i.e. 25 mg./ml.

To determine the degree of inhibition of the toxin, measured by the skin test in adult rabbit (Craig, J. P., Nature 207: 614, 1965), 0.25 mg. of NIH Lot 1 toxin will be mixed with APAs so that the dose of the APAs will be 10, 1 or 0.1 μM in the mixed solution. The mixtures will be incubated at 37°C for one hour. The mixed solution and tripling dilutions of the mixture will be injected intradermally on the back of adult rabbits. There will be two controls - one of the toxin alone and

the other of the APA alone, in each animal injected with the mixtures.

The APAs which inhibit the action of cholera toxin as tested in the skin of adult rabbits, will then be tested in the adult rabbit intestinal loops. For this purpose 5 ligated intestinal loops will be made (De, S. N. and Chatterjee, D. N., J. Path. Bact. 66: 559, 1953). Two kinds of experiments will be carried out in loops thus prepared. In one series the APAs will be mixed with 12.5, 2.5 and 0.5 mg. of toxin and the mixture will be injected into the loops. In each rabbit there will be a positive and negative control. In another series 5 mg. toxin will be injected, premixed and postmixed, into the loops (0.5 mg. of toxin when introduced into the ligated ileal loops in rabbits induces fluid accumulation in 95% of the cases). Besides the positive and negative control the toxin mixtures will be prepared and injected in the following ways (dose of APA will be determined from skin test results):

1. Toxin 5 mg. +APA premixed in buffer and incubated at 37°C for one hour and then injected in the loop.
2. Toxin 5 mg. injected first in the loop, removed immediately and then the APA injected into the same loop.
3. Toxin 5 mg. injected first in the loop, removed immediately and after 5 minutes the APA will be injected into the same loop.

The rabbit will be sacrificed eight hours after the injection of the last sample in each rabbit. The ligated loops will be harvested and fluid volumes and length of loop recorded.

The APAs which behave as significant inhibitors of cholera toxin will be tested in ligated small intestine of rats. In this case three experimental materials, the mixture of APA and toxin, toxin alone and APA alone will be injected in three loops. The harvesting will be carried out on the eighth hour (Nature 220: 814, 1968).

The APAs which behave as significant inhibitors of cholera toxin will also be tested for toxicity in rats. For this purpose different doses of APAs will be administered orally to young rats (100 ± 10 gms.). The weight

and survival of the young rats will be noted.

C. Significance

Toxin inhibitors of known molecular structure may give a clue to the mechanism of action of cholera toxin. It is also important to find out if any of the aromatic polysulphonic acid inhibitors can stop the action of cholera toxin after the intestine has been exposed to the toxin.

D. Facilities

We have all the facilities to carry out the skin and loop tests mentioned in this protocol. The major expenditure will be the procurement of rabbits. Clippers for shaving experimental areas of rabbits have already been ordered. More aromatic polysulphonic acids will be procured. Other chemicals and supplies have been ordered.

E. Supporting Data

Several aromatic polysulphonic acids have been found to be effective inhibitors of staphylococcal α-toxin by Arbuthnott and co-workers (Biochem. J. 108: 41-48, 49-55). A few APAs have also been found to be effective inhibitors after mice have been exposed to the staphylococcal α-toxin.

Preliminary studies in our Laboratory indicate that at .982 μM and 1.17 μM aromatic polysulphonic acids No.IX and XVIII respectively are significant inhibitors of cholera toxin (.025 mg. in rabbit skin) and that several others are partially effective.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
R. A. Phillips	Director	5	71,184	3,559
K. M. S. Aziz	Sr. Investigator	25	52,944	13,236
A.M.M. Mohsin	Res. Associate	40	19,332	7,733
S.Z.A. Quader	Veterinarian	40	10,764	4,306
G. K. Bhuiyan	Res. Assistant	50	8,388	4,194
J. Rashid	Lab. Assistant	50	5,664	2,832
A. Jalil	Lab. Assistant	50	3,096	1,548
Supplies	Rabbits 300 @ Rs 70/rabbit			21,000
	Rats 400 @ Rs 6/rat			2,400
	Surgical supplies, Aromatic Polysulphonic Acids, other chemicals, drugs, plastics, needles, syringes, laboratory glassware, etc.			12,000
Travel	None			
Publication costs				2,000
All other expenses	Repair and maintenance			1,000
	Secretarial			500
				76,408
Overhead 30%				22,922
Total				99,330

PROTOCOL CODE 07 70 01

SECTION I

IRON ABSORPTION IN SYLHET TEA GARDEN

Jon E. Rohde
Donald Mackay

SUMMARY

Clinical refactoriness to oral iron has characterized a number of severe hypochromic anemias in the Sylhet Tea Estates. The ability of a selected few of these patients to absorb an Fe^{59} labelled ferrous salt will be investigated as a preliminary survey to detect hitherto unreported iron malabsorption.

RESEARCH PLAN

In spite of the generally high level of iron present in the average Indian diet (12-30 mgm daily[1]) hypochromic anemia is a widespread disease on the subcontinent. Maternal blood loss, parasitism and other dietary deficiencies are frequently etiologically related to the anemia[1]. However, in a large group of patients demonstrating adequate iron intake, non-absorbability of the dietary iron due to complexing[2,3] has been shown to be the cause of the deficiency. All of these patients show normal absorption when presented with a ferrous salt load[2]. Except in cases of severe steatorrhea, mucosal malfunction and iron malabsorption per se have not been considered a recognized etiology of hypochromic anemia.

That malabsorption of xylose, vitamin B 12, and folate occurs during non-specific gastrointestinal bacteria infection has been amply demonstrated in this laboratory[4]. There is some evidence to suggest that through repeated insults, the normal village East Pakistani small bowel undergoes morphologic and functional changes suggestive of early chronic tropical sprue, in the absence of any overt malabsorption or steatorrhea. The observation of Dr. D. Mackay that many of his severe hypochronic anemias (hgb <5 mg %) respond poorly or not at all to oral iron but demonstrate a brisk rise in hemoglobulin when given parenteral iron suggested the possibility of true iron malabsorption in these cases. Studies of tea garden workers in Assam and Ceylon have not reported this phenomenon[1]. We propose here a preliminary survey of several selected anemic patients designed to see whether this malabsorptive condition actually exists.

Methods

Patients, preferably male, under the care of Dr. Donald Mackay in the Sylhet Tea Estates will be candidates for the study. Six to eight patients exhibiting a typical hypochromic microcytic peripheral blood smear with no other cause for anemia (HgbS or folate), free of significant parasitism, with a history of less than 3 gm Hgb response to a four week course of oral iron therapy will be chosen for study. Body weight and height, stool exam, hematocrit and hemoglobin will be measured. Oral intake will be

limited to water for four hours prior to the administration of the test dose 3.5 mgm of ferrous ascorbate labelled with 5 uci Fe^{59}. It has been well demonstrated virtually 100% of an absorbed iron load will be found in the red cells by fourteen days after administration[6]. Therefore, two weeks later, 15 c.c. of venous blood will be taken for repeat hematology and measurement of r.b.c. radioactivity by gamma counting. Body composition data[5] will be used to estimate red cell volume and per cent iron absorption computed by:

$$\% \text{ absorbed} = 100X \frac{\frac{Fe^{59} \text{ cpm/cc whole blood}}{Hct} \times \text{total rbc vol.}}{Fe^{59} \text{ dose cpm.}}$$

Interpretation

Iron deficient subjects have been shown to absorb 19-60% (mean 44%) of an administered ferrous salt load while non-anemic controls absorb 2-12% (mean 7.2%)[2] thus we would expect these patients to absorb greater than 20% of the administered dose of Fe^{59}, averaging much higher than this base value. Absorption of 15% or less would be construed as definite evidence of malabsorption of free ferrous iron. In the event of this finding, a more thorough study involving marrow, plasma iron, double labelled iron techniques, Xylose absorption and intestinal biopsy will be proposed to rigorously delineate the parameters effecting the defect.

Appendix

Dosimetry: Assuming 100% absorption of 5 uCi Fe^{59} 560 milli rads will be administered to the blood (organ of maximal exposure). This is about 12% of the acceptable yearly exposure as recommended by the USAEC and International Radiation Health Safety Committee.

References

1. Foy, H; Mondi, A. J. Trop. Med. & Hyg. Feb 1958, p. 3.

2. Hussain, R; et al. Am. J. Clin. Nutri., 16 464 1965

3. Layrisse, M., et al. Am. J. Clin. Nutri., 21 : 1175, 1968.

4. Lindenbaum, J. Am. J. Clin. Nutri., 21 : 1023, 1968.

5. Rahman, M. PSCRL - paper in preparation.

6. Josephs, H. W. Blood, 13 : 1, 1958.

SECTION II

DETAILED BUDGET

Personnel	Title	% Time	Rupees Annual Salary	Rupees Total
Jon E. Rohde	Research Physician	10	-	-
Donald Mackay				
Labor	Hematology			100
	Isotope preparation and counting			50
Supplies	Fe^{59} (on hand) Syringes containers, etc.			100
Travel	4 round trips 2 days each to Sylhet @ Rs 97.50			390
				640
Overhead 30%				192
Total				832

PROTOCOL CODE 08 70 01*

SECTION I

DEMOGRAPHIC STUDIES IN RURAL EAST PAKISTAN

Mahbuddin Ahmed, Dacca University

Alauddin Choudhury
Wiley H. Mosley

SUMMARY

The demographic studies in the Matlab vaccine trial area will be continued for 4 years (FY 70 - FY 73) as a collaborative project between the Institute of Statistical Research and Training of the University of Dacca and the Pakistan-SEATO Cholera Research Laboratory.

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*Information for PSCRL reference only. The project proposal is being submitted to USAID by Dr. Ahmed for funding.

RESEARCH PLAN

Background

The decennial population censuses of East Pakistan provide a basis of computing a growth rate at intervals of ten years and this basis is questioned too often by the experts to be of any value to the users. Besides, these censuses do not provide any insight into the dynamics of population growth. No reliable and efficient system of recroding birth, death and migration is functioning as yet in East Pakistan. The Population Growth Estimate (PGE) experiment of Pakistan, which was meant to provide information on population growth was discontinued after 1964. The Population Growth Survey (PGS) undertaken by the Central Statistical Office (CSO) after discontinuation of the PGE, has yet to validate the data they obtain and produce on population growth. Very little was known about the demographic characteristics of the people of East Pakistan either from official or unofficial sources, until in 1966. At this time Pakistan-SEATO Cholera Research Laboratory (PSCRL) of Dacca began to collect and process information on the births, deaths, and migrations that occurred in 132 villages in the Cholera Vaccine Field Trial areas of the Matlab Bazar Thana in the Comilla District, East Pakistan.

Reports of the basic demographic features of the survey population for the years 1966-67 and 1967-68 have been published by the Pakistan-SEATO Cholera Research Laboratory, and the 1968-69 report is in preparation. The population of the field trial area, roughly numbering 220,000, are under continuous observation by a team of trained field staff who record and report any vital event that occurs in every household in the area.

When cholera has been brought under control in the future it may well be possible that cholera research in the area will lose its urgency and importance. It is extremely important that the demographic aspect of the vaccine trial area be continued even though cholera research in the area may be discontinued.

The continuation of the demographic study can be easy and convenient if a collaborative venture is developed between the Institute of Statistical Research and Training (ISRT) of University of Dacca, which has

on its staff, skilled and competent demographers and statisticians, and the Pakistan-SEATO Cholera Research Laboratory. Collaboration of the staff of the two institutions would provide a basis for the eventual use of this area as a study area for the Government of Pakistan, with all of the data of the excellent preliminary work done by the Pakistan-SEATO Cholera Research Laboratory accruing to Government.

In the vaccine field trial area in Comilla District, the PSCRL has provided the population with immunizations against cholera and facilities for the treatment of purging diarrheas, which may have resulted in a reduction of mortality. Another result of their efforts has been the establishment of a cordial relationship with the people of the area which has a direct bearing on the ready cooperation of the public in the collection of demographic data.

In addition to the collaborative venture with the PSCRL it would be useful to have a control area with a similar organization set up under ISRT surveillance, but without treatment and preventive services, and to make a comparison of the results. In order to make a valid comparison it will be necessary to have a control area of approximately 60,000 persons.

In order to conserve human and financial resources it is proposed that four Union Councils of the Chittagong Division related for the Vital Registration Demonstration Project of the Family Planning Council, be used to serve as the Control area.

Objectives

It is proposed that the demographic studies of PSCRL be continued and similar studies be set up in a control area as suggested above. The general objectives of the project may be outlined as follows:

1. To fill up the gap in population studies in rural East Pakistan.
2. To study the mortality pattern of a rural population, including infant mortality, maternal mortality, and the mortality differentials.

3. To study the fertility pattern, including marital fertility, the determinants of fertility and fertility differentials.

4. To study the extent and patterns of migration, and the causes of migration.

5. To study long-term annual trends in fertility, mortality, and migration, and the factors that influence them.

6. To study child spacing, pregnancy intervals, age parity distribution of births, age at marriage and other factors related to fertility.

7. To evaluate the cost and efficiency of this intensive longitudinal method of demographic study, and make recommendations for a workable and economically feasible national system.

8. To serve as a complimentary case study for studies to be undertaken by other institutions who are trying to develop such national systems for population studies in East Pakistan.

Methods

The Cholera Vaccine Field Trial area in Comilla District, East Pakistan has been chosen as the main area of the study, because of the vast amount of supporting data already available about this population of 220,000, and because of the excellent rapport that has been already established in the PSCRL with the people of the area. As has been already mentioned in the statement of 'Background', that it is necessary to have a control area for this study. For a useful and valid comparison, the four Union Councils of Vital Registration Project are proposed as the control area. The Vital Registration sample Unions of the Chittagong Division, have been chosen on the grounds of accessibility from Dacca, and similarity with main study area. The main purpose of overlapping the control area of this study with a part of the Vital Registration Study area is to take advantage of the preliminary operations such as mapping and houselisting, and to provide a basis of comparison of the efficiency of the two methods of study.

The general surveillance procedures which have been developed, and are being carried out, in Vaccine Field Trial area will be continued and followed. The main features of this procedure are as follows:

1. A census of the study population is taken with information on name, age, sex, household size, marital status, occupation, etc.

2. Household cards are prepared and maintained in each household.

3. A Population Register is prepared and maintained.

4. Births, deaths, and migrations are reported on precoded standard forms.

5. Field surveillance is maintained daily be a village 'dai' (Lady Field Worker) recruited locally, who visits every household of her area daily.

6. A Field Assistant visits every house once a week accompanied by a 'dai' and signs the household card and completes the birth, death, and migration forms as and when necessary.

7. An Inspector visits the field assistant at least twice a week and gets reports from the latter. The inspector visits every house at least once a month, signs the household card and checks on the completeness of birth, death and migration registration.

8. Above the Inspector, there is a Field Surveillance Assistant who spot checks areas in every village at least once a month.

9. Over this entire group of workers is the Section Chief of the Field Surveillance Section and the Field Surveillance Supervisor who coordinate the field activities, schedule the surveillance program, check on the completeness of the birth, death and migration forms and followup any discrepancies. They are assisted by the clerical staff.

10. There is a daily messenger service between the Dacca offices of the PSCRL and the field office.

The ISRT staff consisting of the Project Leader, Research Advisors, Statisticians, Research Officers, Research Associates, Research Assistants will analyze the data collected in the Vaccine Trial Area continnuously with the help of a Computer available in Dacca.

Basic demographic rates and ratios will be prepared and published annually in a report form similar to that published by the Pakistan-SEATO Cholera Research Laboratory in 1967 and 1968. In addition, special studies on demography and methodology will be made by the scientists directly involved in the project and by the external participating scientists.

Sample surveys will be undertaken and by means of a matching analysis, the coverage, bias and reliability of cross-section survey methods in demographic investigation will be evaluated.

The constant and intensive surveillance being carried on in the Vaccine Trial Area will facilitate a complete reporting of vital events. This will provide a basis to test the validity of the approximate techniques of indirect computation of the fertility and mortality indices developed by demographers for use in countries lacking reliable and adequate demographic statistics.

Significance of this Project

This project will provide the first longitudinal study of this depth and scope covering a rural population in Pakistan. With the inclusion of the original PSCRL data the study will cover a period from 1966 to 1974, a period of 8 years of observation. This intensity of observation of the vital rates and characteristics of the rural population will provide time trends analysis which takes maximum advantage of data already collected by the PSCRL. The addition of a control area will measure the impact, of any of preventive and curative activities on death rates in the PSCRL area, and also will provide a comparison of the impact of fertility control in the birth rates of two non contiguous rural areas. Migration can also be measured, and its influence in rural communities determined.

The information gained will be of the utmost importance in measuring demographic change over time in the rural areas of East Pakistan.

The other significant factor will be to measure in terms of cost and validity the method of conducting longitudinal studies of this scope and intensity. The application of this knowledge will be helpful in designing future studies on demographic measurement.

Facilities available to the Project

The physical facilities available at the PSCRL include 12 speedboats with motors and spare parts, and office and storage space at Matlab Bazar equipment for compilation of data in Dacca which includes an IBM card punch, a verifier, and a sorter counter.

The physical facilities at the ISRT include office space, five IBM card punches, two verifiers, and an IBM 101 Tabulator.

Arrangements will be made by the ISRT for use of a computer.

Facilities other than physical, include administrative support and accounting services at the ISRT and PSCRL, the services of participating scientists of PSCRL and Dacca University, including those who will not be paid from the project funds.

Collaboration

This project will combine the resources of the Department of Statistics and the Institute of Statistical Research and Training of Dacca University, the Pakistan-SEATO Cholera Research Laboratory, and the Vital Registration Project of the Pakistan Family Planning Council. The Project Leader will be closely allied to demographic work in all of the above activities, and will meet from time to time with interested parties from all agencies concerned. A maximum of inter-communication will be constantly maintained by the Project Leader in order that all those involved in the project will be appraised of the progress of the project.

SECTION II

DETAILED BUDGET

(for information only)

A. Personnel - Dacca University

Position	Pay and Allowances	Number of Employees	Total Monthly Salary (Rs)	Proportion of Time	Total Annual Salary (Rs)
Project Leader	600 (part time)	1	600		7,200
Research Advisor	300 (part time)	2	600		7,200
Research Associate	250 (part time)	2	500		6,000
Research Officer	450-1050	2	1,400	1.00	16,800
Research Assistant	400-900	2	1,000	1.00	12,000
Machine Operator	300	3	900	1.00	10,800
Field Surveillance Supervisor	1000 + 30%	1	1,300	1.00	15,600
Field Surveillance Assistant	500 + 30%	1	650	1.00	7,800

A. Personnel - Dacca University (continued)

Position	Pay and Allowances	Number of Employees	Total Monthly Salary (Rs)	Proportion of Time	Total Annual Salary (Rs)
Sanitary Inspector	400 + Rs.100	4	2,000	1.00	24,000
Field Assistants	200 + Rs.100	8	2,400	1.00	28,800
Dais	50	50	2,500	1.00	30,000
Administrative Officer	700	1	700	1.00	8,400
Stenographer-cum-Secretary	400	1	400	1.00	4,800
Messenger	100	2	200	1.00	2,400
Country Boatmen	Temporary		1,000	0.50	6,000
					187,800
7½% for annual increments					14,085
Total					201,885

B. Personnel - PSCRL (prior to July 1969 re-org.)

Position	CRL Scale & Allowances	Number of Employees	Total Consolidated Monthly Salary	Proportion of Time	Total Annual Salary (Rs)
Section Chief, Field Survl. Section	B + 30%	1	3,359.80	0.30	12,095.28
Field Surveillance Supervisor	G + 30%	1	1,276.60	0.30	4,595.76
Field Surveillance Assistants	J+Rs 100	3	1,995.00	0.30	7,182.00
Sanitary Inspector	K+Rs 100	14	7,933.00	0.40	38,078.40
Field Assistants	M+Rs 100	52	17,900.00	0.50	107,400.00
Dais	Temp. a/	289	11,956.00	0.50	71,736.00
Clerks (Matlab)	K	3	1,238.00	0.30	4,456.80
Speedboat Drivers	M	14	3,832.00	0.20	9,196.80
Messengers	N-O	2	600.00	0.20	1,440.00

a/ Rs 35-60/month

B. Personnel - PSCRL (prior to July 1969 re-org.) (continued)

Position	CRL Scale & Allowance	Number of Employees	Total Consolidated Monthly Salary	Proportion of Time	Total Annual Salary (Rs)
Country Boatmen	Temp. b/	(variable)c/	3,642.00	0.50	21,852.00
Head, Statistical Unit	F	1	1,292.00	0.50	7,752.00
Statistical Assistants	I-J	5	3,242.00	0.50	19,452.00
Statistical Research Assistant	I-H	1	621.60	1.00	7,452.00
					312,689.04
7½% for annual increments					23,451.63
Total					336,140.67

b/ Rs 100/month

c/ 10-15 in dry season, and 70-80 in monsoon season

PROJECTED BUDGET
(for information only)

Budget FY 1970	Rupees
A. Personnel	
1. PSCRL	336,141
2. ISRT	201,885
B. Supplies and Equipment	
1. Equipment	
a. Long carriage typewriters (2)	3,000
b. Bicycles (2)	600
c. Office furniture	4,000
2. Supplies	
a. IBM cards 500,000	12,000
b. Forms and binding	3,100
c. General office supplies	1,000
C. Computer Time	
1. 100 hours @ 460 rupees/hour	46,000
D. Travel	20,000
Total	627,726
Overhead 6%	37,664
Contingency 7%	43,941
First Year Total	709,331
FY 1971 First Year + 7%	49,653
Total	758,984
FY 1972 Second Year + 7%	53,129
Total	812,113
FY 1973 Third Year + 7%	56,848
Total	868,961

	Rs.
	708,326
	757,909
	810,963
	867,730
Total for 4 years:	3,144,928

Note:

Payable to PSCRL (subject to revision)

	Rs.	
1970	336,141	
10%	33,614	
FY 1970 - Total	369,755	1.
FY 1971 1st Yr. + 7%	25,883	
Total	395,638	2.
FY 1972 2nd Yr. + 7%	27,695	
Total	423,333	3.
FY 1973 3rd Yr. + 7%	29,633	
Total	452,966	4.
Total for 4 years	1,641,692	

PROTOCOL CODE 08 70 02

Report on the Nutrition Program being undertaken at the PSCRL

Robert A. Phillips, M.D., Director

On June 30, 1969 the Director of the Laboratory signed a contract with the Health Services and Mental Health Administration (HS&MHA) of the U.S. Department of Health, Education and Welfare, Public Health Service, Bethesda, Maryland, to undertake studies of nutrition to determine how much wastage of the protein there is in the rice supplied by the U.S. Agency for International Development to East Pakistan peoples due to malabsorption and parasitism. Identical studies will be carried out in Iran and Puerto Rico by other laboratories under similar contracts with the HS&MHA.

We are proposing a shorter, easier and more aesthetic procedure than that in the accompanying protocol. Through an orogastric tube the patient's gut is perfused with an electrolyte solution at the high rate of two to four liters per hour. When a steady state is obtained a bolus containing a colored marker and tagged non-metabolized amino acid will be injected. Stool collection would be limited to that dyed by the marker but urine collection would begin with injection of the bolus and continue for a longer time. From counts of stool and urine the fraction of amino acid absorbed could be calculated. Initial studies indicate that 100% of a bolus of xylose can be recovered from urine and stool.

An additional possibility (suggested by Dr. Jon Rohde) of estimating malabsorption depends upon the measurement of the absorption of the labelled amino acid by a biopsy specimen of the patient's intestinal mucosa mounted in a Ussing chamber. Biopsies with a Crosby capsule could be taken on admission and at intervals after the patient was put on an ideal diet.

The Laboratory also will attempt to determine the protein wastage due to parasites. Initially ascaris will be studied. It is known from the literature the number of eggs produced per ascaris per unit of time. However, the amino acid content of a single ascaris egg is not known. In the lavage studies referred to above the ascaris will be washed out of the patient and the number counted. It will thus be possible to get the average population per individual in the Pakistan population. The Laboratory hopes to persuade Dr. Paul Hamilton to determine the amino acid content of ascaris eggs. It should thus be possible to determine the amount of various amino acids present in the patient's diet which are wasted by ascaris infection per unit of time.

(PROTOCOL)

MEASUREMENT OF NUTRIENT WASTAGE FROM MALABSORPTION IN RURAL INHABITANTS IN PUERTO RICO, IRAN AND EAST PAKISTAN

For this study abnormal protein and fat loss will be measured by the balance technique in selected subjects. The following protocol has been devised as the result of consultation with several authorities and is now submitted for final scrutiny and comment. If agreed upon, work should begin by January 1, 1970.

A conference will be held in the spring in either Dacca or Shiraz, at which time problems arising as the result of actual experience can be discussed and solved, with modifications in the protocol if indicated.

It is essential that each Center follow the basic protocol in identical fashion. Modifications and extended research beyond this are expected and welcomed but <u>are not</u> part of the AID requirement outlined in the basic contract.

Additional material will be sent to you consisting of reprints of methods, background material, etc.

Patient Selection. In each of the three areas to be surveyed, one hundred people of both sexes between the ages of 16 and 50 will be selected at random from a rural area. They must be all of the same socio-economic status and carrying on their normal rural occupations. Persons should be omitted from the study who have evidence of chronic or acute illness, such as any chronic infections, hepatic, cardiac or renal disease or any acute febrile illness. Also excluded are women currently pregnant or recently delivered, and any person recently or currently being treated with vitamins or antibiotics over more than 10 days' time. The persons studied therefore will be representative of the average population. They will be admitted to the hospital in groups of four at a time.

Investigative Procedure. On the first day (day 0) each person will have a complete history and physical exam. The history should provide dietary information, such as frequency of meat consumption, types of cereal grains eaten, and kinds of vegetables and fruit usually consumed. Samples for the initial laboratory studies are obtained on day 0 or the morning of day 1, and should include the following: complete blood count including blood film, urinalysis, stool guaic test, examination of the stool for ova and parasites, bacterial cultures of blood, urine and stool, total serum protein, albumin, cholesterol, calcium, carotene, blood urea nitrogen, glucose, prothrombin time, serum glutamic oxaloacetic transaminase (SGOT), and alkaline phosphatase. Approximately 15 cc of blood should be obtained, the serum being placed in a folate free tube ("Vacutainer", Becton, Dickinson and Co., Rutherford, N.J.) for subsequent analysis of serum folate, serum B12, serum iron and total iron binding capacity. After

separating serum from cells, freeze for future analysis. Ideally, a bone marrow aspiration should be done on each patient and the smears stained with Wright's stain.

Intestinal absorption tests are to be started on the day following admission (day 1) and the test diet begun.

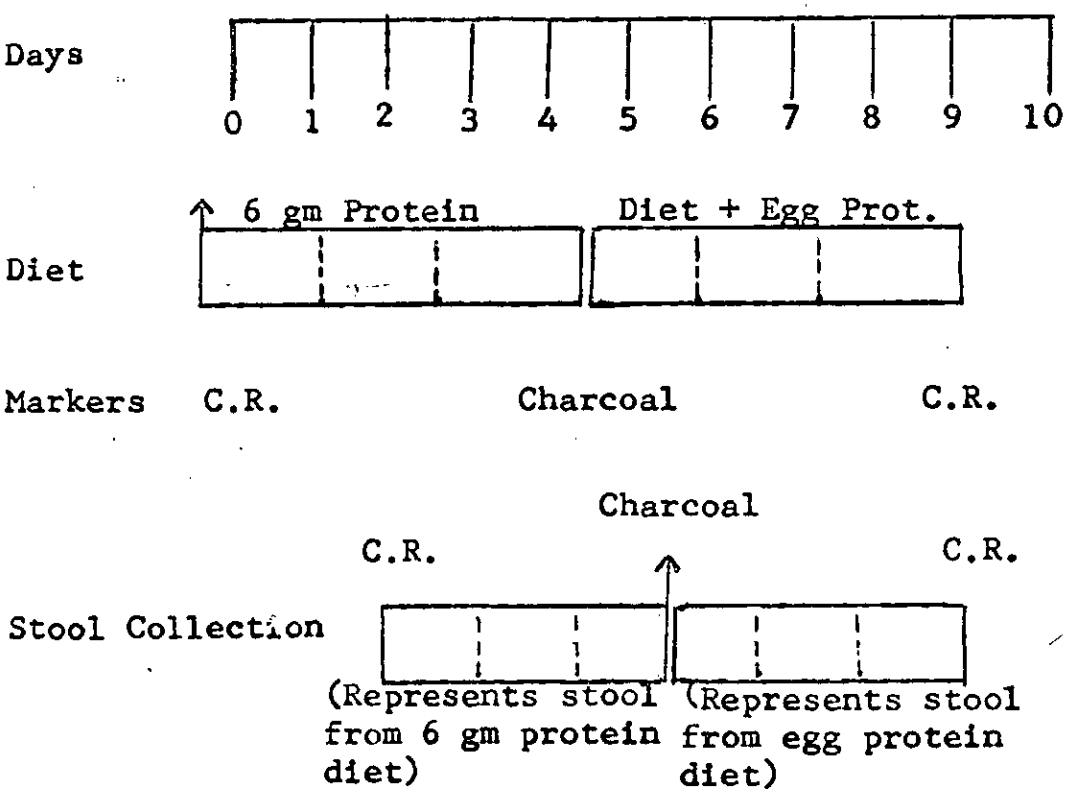
At the beginning of Day 1, the patient is started on the low protein, 84 gm fat diet (attached). At the same time a carmine red stool marker, 1.2 gm, is giving by mouth and stool sampling begun.

Cabalt^{60}-B_{12}, 0.5 mcg, is given by mouth as the first step of the hepatic uptake test for B_{12} absorption. Prior to administration of the isotope, background counts are obtained over the liver.

Day 2. Breakfast is omitted and a d-xylose test is performed by oral administration of 25 gm d-xylose. Urine is collected for 5 hours, during which time the subject is kept well hydrated by the hourly intake of 100 - 200 cc water. Urinary excretion of d-xylose is measured by the photometric method of Roe and Rice.

The remainder of the study period is occupied by measurement of fat and nitrogen balance. As soon as the carmine red marker is seen in the stool, complete collections of stool are made. One gallon paint cans are most conveniently used, with a shaker. Each stool for the 3-day period is placed in the can, the pool being mixed, with aliquots taken for nitrogen and fat determination. After three full days on the low protein diet (at noon on day 4), the diet is then changed by the addition of egg protein (as noted in the attached diet). At this time a charcoal marker (1.0gm) is given by mouth. As soon as the charcoal appears in the stool, the first collection, representing that stool formed during the low protein diet, is stopped

and a new collection begun. After three days on the egg protein diet, a carmine red marker is again given by mouth. Stool is collected until this marker appears again, this sample representing that stool formed during the egg protein diet. A schematating outline of a possible balance situation is shown below.



C.R. = Carmine red

The stool pools are best homogenized by a paint shaker, aliquots being then removed for analysis of fecal fat and nitrogen.

Interpretation. 1) Fat absorption is assessed from the two measurements of fecal fat. Any excretion in excess of 6 gm daily is abnormal, providing the diet contains at least 80 gm fat. 2) Protein absorption is assessed by the increment of nitrogen excretion in the second period as compared to the first. Normally, all egg protein should be completely absorbed and therefore there should be no greater fecal nitrogen during the period of egg protein diet as compared to that excreted during the low protein dietary period. This assumes that the endogenous protein produced during the digestion of the egg protein is also completely absorbed.

Day 8. Hepatic uptake of Co^{60}-B_{12} is measured using surface scintillation counting.

Day 9 (or when the second stool collection is completed). Jejunal Biopsy. (This is delayed until the end of the study in order to avoid possible bleeding which would introduce an error by contributing to fecal nitrogen. Furthermore, it provides for poor cooperation at the outset by giving an unpleasant procedure).

For jejunal biopsy the Crosby capsule is ideal, as it may be passed on the end of a 100 cm length of pliable tubing (intramedic polyethylene tubing, PC 200, Adams) and without recourse to a fluoroscopic screen. After swallowing the capsule, the patient is placed on his right side until bile is seen to drain from the tubing. He is then placed on his back and when 90 - 100 cm of tubing have passed, a flat plate x-ray of the abdomen will localize the tube at the duodeno-jejunal angle, once the tube is gently filled with

hypaque dye. About 20 cc of suction on the tube will fire the capsule, which is then removed and opened. Obtaining the specimen should take 2 - 4 hours. The specimen may then be gently sectioned with a razor blade and a portion immediately wrapped in wax paper, placed in a plastic test tube in dry ice and quick frozen by pouring acetone around the tube. This portion is then stored at -20° for subsequent analysis of disaccharide enzymes. The other portion is gently spread, villi upwards, on a piece of filter paper or blotting paper and immersed in 10% buffered formalin for subsequent histologic preparation (hematoxylin and eosin) and examination. If a dissecting microscope is available, the specimen may be examined grossly and classified as fingers, leaves, convolutions, or flat.

The subject may then be discharged to his village after being placed into one of three categories for daily treatment: (1) placebo; (2) 5 mg of folic acid twice daily with 1000 mcg B_{12} subcutaneously on discharge and every two months; or (3) tetracycline, 250 mg twice daily. A worker should visit the village weekly to ascertain that treatment is progessing as scheduled. At the end of six months appropriate subjects are brought back to the research ward and re-studied. (Details will be worked out after the conference).

LOW PROTEIN DIET (a)

	Food Item	Approx. Measure	Wt. (gm)	Prot (gm)	Cal (Kcal)	Fat (gm)
Breakfast	Orange	1/2 medium	105	0.6	45	0.4
	Apple	2 medium	300	0.5	174	1.2
	Grapes	1/2 cup	76	0.4	50	0.3
	Tea	1 cup	-	-	-	-
	Sugar, white, gran.	1 tsp.	4	0	15	0
Lunch	Beets	1/4 cup	41	0.6	17	trace
	Celery	1/4 cup	33	0.3	6	trace
	Carrots	1/4 cup	36	0.3	11	trace
	Cabbage	1/4 cup	42	0.6	12	trace
	Potato, European	1/4 cup	50	0.7	38	trace
	Onions	1/4 cup	27	0.3	12	trace
	Clarified butter (ghee)	1/4 cup	56	trace	400	44
	Tea	1 cup	-	-	-	-
	Sugar, white, gran.	1 tsp.	4	0	15	0
	Lemon pudding	1 cup	-	0	359	11
Dinner	Egg plant	1/4	100	0.9	27	0.3
	Olive oil	1 tbsp.	14	0	125	14
	Onion	1/4 cup	27	0.3	12	trace
	Celery	1/4 cup	33	0.3	6	trace
	Cabbage	1/4 cup	42	0.6	12	trace
	Carrots	1/4 cup	36	0.3	15	trace
	Salad oil	1 tbsp.	14	0	125	14
	Tea	1 cup	-	-	-	-
	Sugar, white, gran.	1 tsp.	4	0	15	0
				6.7 (1.10 gN)	1491	85.2

tsp = teaspoon (5 ml. or gm)
tbsp= tablespoon (15 ml or gm)

(a) Calculations based on Food Composition tables compiled by Food and Agriculture Organization of the United Nations

Aim

To prepare a natural food diet with a minimal level of protein which provides about 80 grams of fat daily. This daily meal pattern will be fed to subjects in a metabolic ward for the purpose of studying protein and fat absorption.

This will be accomplished by feeding a 6 - 7 gram protein diet first, with stool analysis for nitrogen to obtain the degree of endogenous nitrogen excretion. Following this the same diet will be continued to which is added egg protein as it has been demonstrated that egg protein is absorbed completely. Fat absorption will be determined by stool analysis for fat. (On an intake of between 80 and 100 gm. of fat the daily excretion of fat should not exceed 6.0 gm).

The suggested meal plan can be modified according to the availability of the various food items provided the protein (nitrogen) content does not exceed that suggested. This meal plan has not as yet been tested for palatability, size of serving, nor analyzed for nitrogen content. Presumably, minor adjustments will have to be made in the three Centers. Suggestions are also included for calorie raisers.

For breakfast the fruits can be sliced or sectioned and mixed as in a fruit compote. For lunch the vegetables can be sauted in the 1/4 cup of clarified butter (ghee).

Recipe for Lemon Pudding

1 tablespoon cornstarch
1/3 cup white granul sugar
3/4 cup boiling water
1 tablespoon butter
1/4 cup lemon juice
1/4 teaspoon grated lemon rind
Few grains nutmeg

1. Thoroughly mix the cornstarch and sugar in a saucepan.
2. Add the boiling water to the sugar mixture.
3. Stir over direct heat until the mixture boils for 2-3 minutes.
4. Remove the pan from the heat and add the butter, lemon juice, grated rind and nutmeg.

The first four food items listed under "Dinner" are to be utilized in the following recipe for egg plant. A cabbage salad (celery, carrots, salad oil) is also to be used at this meal.

Recipe for Egg Plant

Peel 1 medium egg plant. Halve it lengthwise and cut the halves into vertical slices about 1/4 inch thick. In a skillet saute the egg plant slices in 1/4 cup olive oil, seasoned with salt and pepper to taste, until they are golden. Set the egg plant aside.

In another skillet saute one onion in 2 tablespoons of olive oil, i.e. chopped, with 1 garlic clove and oregano, and salt and pepper added to taste, for about 10 minutes or until the onions are tender. Stir in one cup of freshly cooked vegetable (e.g., celery) and the reserved egg plant. Simmer the vegetable for about 15 minutes. (This will make 4 servings)

Pure sugar candies and bottled soft drink can be used if necessary for additional calories. 100 g, or approximately 4 candies, contain about 386 calories.

It is imperative that a nitrogen analysis be done on the food composite prior to use. The nitrogen content for a specific food item can vary with its source.

It would also be advisable to include a commercially available one-a-day vitamin capsule, so that the daily vitamin requirements are met. Some of these preparations also include iron and other minerals. The short duration of the experiment will not require a mineral supplement.

Test Protein:

1 medium raw whole egg = 50 gms.
1 medium raw whole egg = 6 gms. of protein

Therefore 6 medium raw whole eggs = 30 gms of protein (4.93 gN). During the Egg Protein diet period give 6 eggs daily - 2 eggs at each meal.

REPORT OF THE EPIDEMIOLOGY DIVISION

Wiley H. Mosley, M.D., Head

Introduction.

There was a major expansion of the activities of the Epidemiology Division into three areas during FY69. The vaccine field trial population was doubled from approximately 120,000 to 230,000 in order to test the effectiveness of monovalent Ogawa and Inaba vaccines and a purified Inaba antigen. Epidemiological field studies extended to Chittagong where there was the simultaneous occurrence of cholera due to both the El Tor and classical biotypes of Vibrio cholerae. The demographic and population studies program was expanded by the inititation of a study of pregnancy prevalence, pregnancy interval and fetal wastage among a group of women in the Matlab field trial population.

Concurrent with the expansion of the field trial area, Matlab experienced the biggest cholera epidemic since the inititation of studies there in 1963. There were over 1,700 confirmed cholera admissions. This permitted the most extensive field evaluation of oral therapy in cholera to date in a collaborative effort between the Epidemiology and Physiology Divisions. Major assistance in these oral therapy studies was provided by Drs. Roger Rochat, Barth Reller, Eli Abrutyn and John Forrest, Epidemiologic Intelligence Service officers from the U.S. National Communicable Diseases Center, Atlanta, Georgia. In the spring of 1969 the Epidemiology Division collaborated with a team of scientists from the Soviet Union in the evaluation of bacteriophage prophylaxis in family contacts of cholera patients.

A brief review of the progress in the Epidemiology Division is presented below. This review will include studies completed on protocols presented in previous

Technical Committee reports and progress reports on new protocols initiated in FY70.

03 70 01 (formerly E/P11) - A Community Study of Inapparent Cholera Infections 1968-69.

This was the third year of intensive serological and bacteriological investigations in village Meharan, the Hindu fishing village of 1,600 persons, in the Matlab area. During the three-month cholera season this village had 12 hospitalized cases from 9 (3.2%) of the 272 families participating in this study. Intensive bacteriologic and serologic studies revealed, however, a total of 115 infections involving 74 (27%) of the families. A breakdown of these 115 infections revealed that 11% required hospitalization, 15% were treated as outpatients, 15% had mild diarrhea and 59% had no symptoms whatsoever. Thus, although 1% of this community required hospitalization for cholera, the infection rate was at least 10%, with an infection rate of 25% in children under the age of 5 years. All cases in the 1968-69 cholera season were due to classical cholera of the Inaba serotype. This study is continuing into the 1969-70 cholera season, and as of December 1969, 32 infections with El Tor Ogawa have been identified. It will thus be possible to compare the epidemiological pattern of classical and El Tor cholera within the same village.

E/P14 - Studies of Cholera Enterotoxin Antibodies and of Cholera Enterotoxin and Toxoid Present in Field Trial Vaccines.

The investigations under this protocol were completed in FY69. The principal observations were the serological studies which suggested that the vascular permeability factor and the ileal loop toxin of Vibrio cholerae were identical. This permits a much broader interpretation of epidemiological investigations utilizing the skin test to measure toxin neutralizing antibody.

03 70 07 (formerly E/P15) - Epidemiological Studies of Cholera El Tor in Chittagong - a Comparative Study with Classical Cholera.

During FY69-70 Chittagong experienced an epidemic of cholera due to both classical and El Tor biotype. This study consisted of bacteriological surveillance for cases in all the hospitals and bacteriological and serological followup of family contacts of confirmed cases and a systematic surveillance of nightsoil and latrines for evidence of community infection with Vibrio cholerae. In summary, these studies revealed a marked difference in the spectrum of infection between classical and El Tor cholera. Among family contacts infected with El Tor cholera only 3% required hospitalization, whereas among contacts infected with classical cholera, 20% required hospitalization. Nightsoil and latrine surveillance revealed that the El Tor vibrio could be isolated from the environment ten times more frequently than the classical vibrio in areas where cases due to both biotypes were occurring simultaneously.

03 70 05 (formerly E/P16) - The 1966-67 Field Trial, Third Year - Evaluation of Booster Injections.

The third year followup of the 1966-67 field trial is shown in Table 1 below.

TABLE 1

1966-67 Field Trial (Third Year) Cases
(Ogawa in parentheses)

Vaccine Year			Age		
1	2	3	0-4	5-14	Total
00	0	0	7 (1)	12 (1)	19 (2)
XX	0	0	20	2	22
X0	X	X	1 (1)	3 (1)	4 (2)
XX	X	X	2	3	5

Vaccine 0 is the control vaccine and Vaccine X is a commercial cholera vaccine, prepared in the United States, containing both Ogawa and Inaba organisms. The first two injections were given in 1966, the third injection in 1967 and the fourth injection in 1968. Again, essentially all cases occurring in the 1968-69 cholera season were due to the Inaba serotype. The ages listed in Table 1 were the ages at the time of vaccination and the population is now, in fact, three years older. The number of participants in all four vaccine groups is essentially identical. It is evident from Table 1 that there was substantial protection in the two groups that received annual booster inoculations. The group that received two doses of vaccine in 1966 and subsequently only placebo injections (Group XX00) showed no protection when all the children ages 0-14 years are taken together. The striking finding, however, is that in the 0-4 year age group, there were significantly more cases in children receiving two injections in 1966 as compared with the control, while in the older children there were significantly fewer cases. It appears then that children who received two inoculations of cholera vaccine, but were no longer boosted, were more susceptible to cholera than children receiving only placebo injections. A possible explanation is that the placebo group has developed "intestinal immunity" from natural infection over the past three years, while the group receiving cholera vaccine in 1966 was transiently protected against intestinal infection and therefore against this route of oral immunization.

03 70 05 (formerly E/P17) - The FY69 Vaccine Field Trial - Test of the Purified Inaba Antigen and of the Lyophilized Monovalent Inaba and Ogawa Cholera Vaccines.

The 1968-69 field trial tested three new cholera vaccines in a new population of 120,000 persons. The vaccines were a whole cell lyophilized monovalent Ogawa vaccine, a purified Inaba antigen and a whole cell lyophilized monovalent Inaba vaccine, with a tetanus and diphteria toxoid control. Only a single dose of of the vaccine was given to children ages 0-14 years. Essentially

all the cases were due to the Inaba serotype of Vibrio cholerae and the results indicated that within the first three months following inoculation the monovalent Inaba vaccine and the purified Inaba antigen gave approximately 90% protection while the monovalent Ogawa vaccine was ineffective. Serological surveys indicated that the degree of protection induced by the Inaba vaccines correlated with the rise in antibody titer. The monovalent Ogawa vaccine did induce Inaba vibriocidal titers; however, it did not protect.

E/P18 - Inapparent Infection in Matlab Children.

In this study a sample of 1,300 children participating in the third year followup of the 1966 field trial was bled four times through the 1968-69 cholera season and rectal swabs were collected twice weekly from October 1968 to February 1969. Preliminary analysis of the data available reveals that there were 5 hospitalized cases among this subsample of children and 15 were found bacteriologically-positive by the twice weekly rectal swabs. Analysis of the serological data indicated that among 652 participants who received the placebo vaccine, there were 121 serological conversions. A striking finding was the fact that 92 of these conversions occurred between the first and second bleedings. Currently it is difficult to interpret these findings. They would suggest that Vibrio cholerae is widely disseminated through the population before significant numbers of cases begin to appear, although the possibility that these conversions were due to some other factor cannot be excluded. This problem is being pursued during the 1969-70 cholera season.

E/P20 - Purging of Family Contacts.

In this study family contacts of cholera patients were hospitalized as soon as the index case was identified. Rectal swabs were obtained and they were purged according to one of several techniques, including mannitol, magnesium sulfate or mannitol following a fatty meal.

The purged stool was cultured and rectal swabs were than obtained for the next four consecutive days. One hundred and eighty-seven contacts were studied, and an overall 50 (27%) were found infected. Only 27 of these positives were detected by purge. The remaining 23 were detected by multiple rectal swabs. Thus purging was not an efficient method for detecting inapparent infection among family contacts of cholera patients.

03 70 04 - Serological Studies of Inapparent Cholera Infections Utilizing the Toxin Neutralizing Test of Craig.

One hundred and six children in village Meharan were intensively followed by daily rectal swabs for 50 days and had three bleedings for toxin neutralizing antibody response. Twenty of these children became infected with Vibrio cholera, 4 requiring hospitalization. On the initial serological survey, 48% of the children had a detectable titer of 3 Craig units or greater. Detectable titers were found more frequently in children ages 2-3, as compared to children ages 8-11. The infection rate was significantly higher in persons with detectable titers; however, detectable titers seemed to be associated with milder symptoms in those persons infected. An antitoxin titer rise of 10 units or greater developed in only 52% of those children infected. Titer rises were more frequently present in persons hospitalized or with diarrhea than those who had no symptoms. In summary, although a rise in toxin neutralizing titer seems to be specifically associated with infection with Vibrio cholerae, the test lacks sensitivity, particularly in detecting inapparent infections.

03 70 06 - Development of an In Vitro Antitoxin Antibody Titration Test.

Relatively pure preparations of toxin have been prepared utilizing the fractionation procedures described by Richardson. This toxin has been used to coat formalized sheep cells for a passive hemagglutination test, utilizing

the microtechnique. Studies indicate that the passive hemagglutination test is specific for the detection of antitoxin antibody and maintains the sensitivity of the In vivo PF neutralizing test. The primary technical problem is the frequent presence of Forssman antibody in normal human sera which apparently can be absorbed by sheep cells prior to carrying out the antitoxin titrations.

08 70 01 - Demographic Studies in Rural East Pakistan.

The third year of registration of births, deaths and migrations in the field trial population has been completed and the vital rates remain about the same, with a birth rate of 47 and a death rate of 15 per 1,000. A special survey to determine the age parity specific birth rates of 30,000 women has been completed. In addition, a study of fetal, neonatal and infant mortality was carried out by matching birth and death certificates over a 12-month period. A project proposal has been drawn up in collaboration with Dr. Mahbubuddin Ahmed of the Institute of Statistical Research and Training, Dacca University, to expand the demographic studies in the field trial area. This proposal has been submitted to U.S.A.I.D. for financing for a 4-year project.

Collaborative Investigations.

A. The Relationship of Cholera Vaccine Effectiveness to Serological Responses in Humans and to the Mouse Protection Test.

This study was carried out in collaboration with Drs. John Feeley and Margaret Pittman. The various field trial vaccines were inoculated into 6-12 month old infants to determine the relative antigenicity of these vaccines based on the vibriocidal antibody response. These results were compared with the relative potency of the vaccines based on the mouse protection test. In general there was a correlation between these two assays, although there

were exceptions to this correlation, particularly with the monovalent vaccines and the purified Ogawa antigen. In comparing the results of these two tests with the effectiveness of the vaccines in the field, it appears that the mouse protection test remains the most satisfactory test for evaluating vaccine efficacy.

B. Bacteriophage Prophylaxis in Families of Cholera Patients.

These studies were carried out in collaboration with a team of scientists from the Soviet Union. Family contacts of cholera patients were visited within one day of hospitalization of the index case and were given orally a bacteriophage preparation or a placebo, consisting only of the suspending medium. They were then followed with rectal swab cultures daily for ten days. Overall, 137 contacts of cholera patients were followed in 28 families. Half of these families were infected with El Tor cholera and half with classical cholera. In summary, the results appear to reveal that bacteriophage had no prophylactic value in preventing subsequent infections or secondary cases in the family contacts.

C. A Study of Pregnancy Wastage and Pregnancy Interval.

This is a collaborative study between PSCRL and U.S.A.I.D. Approximately 200 women in the Matlab area are being followed with intensive interviews and monthly pregnancy tests to determine the frequency of conception and fetal wastage. This study, which began in April, 1969, is a pilot project to determine the feasibility of a large-scale study which will cover 3,000 women in the entire vaccine field trial area. Preliminary results indicate that such a study involving the monthly collection of urine specimens and regular interviews of women for menstrual history and other factors, is feasible. The pregnancy test has been adapted to a microtechnique by this Laboratory, resulting in an 80%

saving in the cost of the reagents. In the first six months of observation, preliminary results indicate that the conception rate is five times more frequent in menstruating women than in lactating women. This study will be continued through FY70 in anticipation of support for the large-scale study in FY71.

Report of the Physiology Division

W. Kendrick Hare, M.D., Head

It is anomalous that a new division of PSCRL, created on July 1, 1969 (the beginning of FY70), should report on events prior to its birth. For this and several other reasons, the report will be brief and will contain generalities rather than detailed and specific data.

There have been several important changes in personnel; three staff physicians have left for higher education, a former research physician, Dr. Ally, has returned after an absence of three years and many technicians have found attractive scholarships abroad. However, the number of personnel is just what it was on July 1, 1968. Dr. Northrup has gone home to NIH and been replaced by Dr. George Curlin who spent three months here in 1966.

For presentation of research accomplishments we have not adhered to the fiscal calendar. For you to appreciate the achievements of the Laboratory we must look back to FY68 and look forward into FY70 and beyond. We will take the forward look first.

Suggestions for Future Research in the Physiology Division.

The Division of Physiology, created July 1, 1969 by the coalescence of several sections, includes the investigators, the staff physicians, the ward, library, animal house, chemistry and clinical pathology. This arrangement relieves the investigators of time consuming administrative chores and permits coordination of research with patient and of animal care. In planning the future of this young Division, we are taking a leaf from the book of Epidemiology Division. Epidemiology has benefited from a long range plan supervised by a consultant who has been in continuous contact with PSCRL for at least five years. Furthermore, the Division Head has been in residence for four years. In contrast, the research related to the individual cholera patient has been

modified annually as a new head of clinical investigation was appointed. The lack of continuity from year to year has been costly in manpower, equipment and productivity. We are now proposing a continuing program for the next few years. As new investigators are recruited and older ones finish their current projects, they will be incorporated into this program.

In order to assure that all new investigators were coordinated with the core program, protocols patterned after the NIH Extramural Research Grant application forms, were introduced. They are reviewed by the senior staff and the investigators, approved or rejected, and those accepted presented to you for appraisal.

The research program is centered around the cholera patient who provides PSCRL with opportunities perhaps unmatched in the whole world.

Cholera in man

The most basic physiological problem for the investigators at PSCRL is the mechanism of action of cholera toxin. The production of toxin by the vibrio has been established and the toxin itself has been separated in almost pure form from the filtrate of vibrio cultures. The result of the action of cholera toxin a massive diarrhea has been recognized for centuries, but a clear demonstration of the process by which the toxin produces the result has not been made. Changes in the transfer rates of sodium and water by the intestinal mucosa in response to cholera toxin have been measured in this Laboratory. These studies in which radioactive isotopes and multilumen intestinal tubes were used have been extended to studies of permeability. Although we find that sodium absorption is impaired in acute cholera, glucose absorption is not different from that in convalescence and this is the basis for successful oral therapy since some sodium transfer mechanisms are interlocked with glucose absorption.

Now that this fortunate association has been exploited we should turn our attention to activities of

the intestinal epithelium that are altered in cholera, and these explorations can be carried out in vitro as well as in vivo. A micro modification of the Ussing chamber will accommodate the small sheet of mucosa obtained with a Crosby biopsy capsule. This greatly increases the opportunities for enquiring into the response of the human epithelium to cholera toxin and into the drugs which may modify this response. The demonstration by Whalen et al that ADH, whether applied to the mucosal surface or given parenterally, increases the net flux of water and salt into the lumen suggests a hormonal factor in clinical cholera, or perhaps it would be more astute to say it suggests an enzymatic mechanism. Cyclic AMP activity can be modified by several drugs, including some that modify adenyl cyclase. It may be that cholera toxin acts at the same site as ADH or that it enhances the action of ADH.

Another approach to the study of the derangement of epithelial function by cholera toxin is through osmolar regulation of intestinal content. We shall hear more this week of our own studies on dogs. These preliminary studies might be transferred to man by the use of perfusion techniques, such as the open ended segment or whole gut procedure.

The colonization of the intestinal tract by Vibrio cholerae is not yet fully described and the volunteers (in the US) may be ideal sources of information on this phase of cholera. If we undertook an investigation of this problem we would have to depend upon early study of contacts with known cholera cases, at best a random method with a scant harvest. The established cholera infection we have in abundance and it is known that in early phase of clinical cholera the vibrio population of the jejunal content may equal that in the stool. Both jejunal aspirate and stool support the growth of the vibrio and the production of toxin. Most laboratory studies of toxin production are carried out at room temperature and in fully aerated media; in the jejunum the temperature is 37°C and the $p^{O}2$ is 25 mm Hg. We are now prepared to compare the toxins produced under these

two conditions using jejunal aspirate as the culture medium.

Preliminary studies indicate that cholera stool supports vibrio growth less well than does jejunal juice. This may be caused by the enzymatic alterations of the jejunal content as it progresses along the intestinal tract, absorption of substrates or to the addition of antibodies to the stool. The immune response of the cholera patient is of great importance and the possibilities for research within this aspect of cholera are enormous.

PSCRL cannot provide facilities and personnel for pursuing all conceivable and promising ideas related to cholera. The relative merits of chemotherapeutic and antibiotic agents, the secondary effects of the disease and its complications can easily consume all our resources. It seems more rational to formulate a plan for at lease five years centered about the effects of the toxin on the intestinal epithelium. The enquiries should be focused on the enzymatic, hormonal and immunological factors that modify the absorptive mechanisms.

The Use of Experimental Animals in Cholera Research

Since cholera does not occur in animals other than man, infection or intoxication must be induced by introducing the vibrio or its products into the gastrointestinal tract. East Pakistan offers no advantage in this approach except for the supply of dogs and monkeys and material from cholera patients. As a matter of fact, we work in this geographic area at a disadvantage because of the shortage of highly trained investigators, the difficulty in obtaining supplies and equipment and delays in communication. The seasonal pattern of the cholera epidemic leaves several months out of each year with only a rare patient and this period can be used for the assay in animals of material collected from the patients and stored. Experimental animals are also necessary for developing new procedures of investigation that if productive and refined, may be used on the patients.

These preliminary studies offer the great advantage of assessing the quality of support from the laboratories for personnel, facilities and performance. We have no specific in vitro test for cholera toxin and since a quantitative measurement is required for interpretation of many experiments, colonies of mice, rats, rabbits, guinea pigs and dogs must be maintained. Chickens, goats and sheep are required for production of antibodies and as a source of red blood cells.

Recommendations

1. The research program should be centered around the cholera patient and should be planned for a five year project.

2. Three senior and experienced investigators should be on the staff on a long term basis: a physiologist, an immunologist and a bacteriologist. It is unprofitable for young, inexperienced physicians to plan and carry out several short term projects on their own initiative. Matured investigators would add continuity and coherence to the program.

PRESENTATIONS

To show this Committee the achievements since your last meeting we have gone back into FY68 and selected studies that have continued into FY69 and even into FY70. Emphasis has been placed on the progress within a research project rather than upon individual protocols.

	Refer to Protocol No.	Subject	Speaker
1.	CI/P8-13	Intestinal immunology	Dr. Mosley
2.	CI/P23	Bacteriophage in cholera therapy and prophylaxis	Dr. Phillips
3.	04 70 03 04 70 07	Oral therapy in cholera	Dr. Nalin
4.	05 70 01	Natural and synthetic inhibitors of cholera toxin	Dr. Aziz
5.	04 70 06	Interaction of neutralizing agents with cholera toxin	Dr. Cash
6.	04 70 04	The colon in cholera	Dr. Rahaman
7.	04 70 02	Effect of cholera on body composition	Dr. Rahaman
8.	04 70 09	Kidney function in cholera	Dr. Cash
9.	04 70 08	Interaction between man and Vibrio cholerae	Dr. Curlin Dr. Barry

	Refer to Protocol No.	Subject	Speaker
10.	04 70 01	In vitro studies in ion movement in human jejunal mucosa	Dr. Rohde
11.	04 70 05	Osmolar regulation of intestinal fluid	Dr. R. Hare
12.	07 70 02	Iron absorption in Sylhet Tea Garden	Dr. Rohde
13.	CI/P31	Age specific electrolyte composition of cholera stool	Dr. Bart
14.	04 70 10	Body composition in cholera patients	Dr. R. Hare

No.3 (04 70 03 & 04 70 07 - Oral therapy in cholera)

Oral maintenance therapy for cholera was introduced 1½ years ago at this Laboratory with the demonstration of an 80% reduction in intravenous fluid requirements in adult patients with severe cholera. They receive an oral solution after correction of shock with intravenous fluids. The practicability of the method in the field was subsequently shown in a trial at Matlab Hospital. Even though the staff was small relative to the patient load, a 70% reduction in intravenous fluids was demonstrated in a series of over 100 patients. The solution used at Matlab has been continued as part of routine treatment so that the total number of patients treated there with this method is now over 1000.

The basic oral therapy method has changed a little since the initial report except that nasogastric tubes have been increasingly used for delivery of the solution in cases requiring large volumes. The formula has been modified in several ways in order to expand the usefulness of the solution. In a practical sense the most significant of these modifications is an increase in the potassium content of the solution from 15 to 25 mEq/liter. Studies in a series of children and adults have shown that the high potassium solution maintains patients in all age groups satisfactorily. A single high potassium solution will simplify management of large epidemics.

A second series of over 30 patients in Dacca has been treated with a solution containing glycine or glycine plus glucose. The studies have shown that the solution with glycine is absorbed and that the addition of glycine to glucose results in a further increases in absorption and a decrease in duration of diarrhea in oral therapy patients. Preliminary results of a field trial of the glucose plus glycine solution on 80 patients at Matlab confirm the decreased duration of diarrhea noted in the Dacca studies.

One of the potentially most important oral therapy studies was carried out on a small series of patients

treated under carefully controlled conditions without any intravenous fluid. Control patients were observed without any therapy for six hours during which time their status remained the same on admission. Patients in both groups were severely dehydrated, acidotic and moderately hypotensive. Blood pH of the patients receiving oral therapy was corrected within three hours and a plasma specific gravity was corrected within six hours after beginning treatment. This study shows that mild to moderately severe cases can be treated with oral therapy alone and suggests that early oral therapy, even in the most severe cases, might obviate the need for intravenous fluids.

No.4 (05 70 01 - Natural and synthetic inhibitors of cholera toxin)

Sera from adult or infant conventional rats or from germ free rats of various strains were all potent inhibitors of cholera toxin. 0.5 ml of serum from conventional rats or from germ free rats neutralized 25 mg of NIH Lot 001 toxin. Another rat serum had a higher inhibitory potency. Complete neutralization of NIH Lot 001 toxin was achieved only in the rabbit small intestine loop assay of the rat serum and toxin mixture. In the rabbit skin assay of the mixture, the toxin is not neutralized or only partly so. The inhibitory potency of rat intestinal wash was much less than the rat serum. The inhibitor in the rat serum was non-dialyzable and heat stable (100°C for 1 hour). Several aromatic polysulphonic acids were tested for their effect on cholera toxin. Preliminary studies indicated that two of the compounds tested are inhibitory.

No.5 (04 70 06 - Interaction of neutralizing agents with cholera toxin)

The factor in cholera toxin responsible for the diarrhea can be completely removed by pretreatment with a number of agents. Kaolin detoxifies a cholera culture filtrate when premixed with it but in a clinical trial

proved to be ineffective. Thus far no agent has been found which can reverse the effect of toxin once it has come in contact with the gut mucosal surface. A number of agents, such as immune serum, aluminum hydroxide, dextran and CPC has thus far been tested.

No. 6 (04 70 04 - The colon in cholera)

An explanation for the difference in electrolyte composition of cholera stool in adult and pediatric patients was sought by studying the transit time from duodenum to anus. It was believed that a longer transit time would allow the cholera stool to be acted upon by the colon for a longer period thereby changing the concentration of Na^+ and K^+. It was, however, found that the transit time in active cholera is extremely variable even in the same patient. Although nearly 14 patients were studied the results did not yield a conclusive finding. It was therefore, decided to confine the study to the colon only by perfusing a solution whose composition was similar to that of ileal fluid found in active cholera.

The study with a slow rate of perfusion (250 mls/hr) showed a decrease in the concentration of Na^+ in the majority of recovered children during the transit through the colon. There was also decrease in concentration of Cl^- but an increase of HCO_3^- which reached a value above 40 mEq/liter, almost similar to the values found in cholera stool. The value of K^+ did not seem to increase significantly. This study has been carried out successfully in patients during acute cholera and will be repeated after recovery.

No.7 (04 70 02 - The effect of cholera on body composition)

An assessment of dehydration in cholera was carried out retrospectively in children by taking careful weight and measuring plasma protein concentration, volume and hematocrit on admission and repeating these after recovery, along with the measurement of bromide space which gave an approximation of the extracellular fluid. It

was found that initial loss of weight can be accounted for mostly by loss from the extracellular space when calculation was made on a retrospective basis. It was also observed that measured plasma volume on admission in some patients gave values which were far below those found on the basis of retrospective calculation after recovery.

No.8 (04 70 09 - Kidney function in cholera)

Though renal failure has always been considered a major complication of cholera, there are no adequate reports in the literature evaluating renal function in the cholera patient. At PSCRL, in fact, renal failure in cholera has been seen in only 4 cases out of the thousands treated. This study is designed to define some of the basic renal function tests in the cholera patients. Preliminary results indicate that glomerular filtration rate increases with time - returning to normal within the first 12-18 hours. There is also a remarkably low titratable acidity considering the severe acidosis of some of the patients.

No.9 (04 70 08 - Interaction between man and Vibrio cholerae

The growth of 569B (Inaba) in jejunal aspirate and in stool from cholera patients, and in peptone water has been compared. The growth rate was estimated from a

linear calibration curve prepared by plotting optical density against colony counts. The inoculum chosen produced a population of 1 x 10^6 vibrio/ml medium; colony counts showed a range of 8.5 x 10^5 - 1.5 x 10^6/ml. The cultures were incubated aerobically at 30°C in a shaking water bath or aerobically and anaerobically at 37°C. The maximum yield in peptone water under the anaerobic conditions was about 5 x 10^8 as compared with 1 x 10^9 aerobically. In the logarithmic phase, the generation time was 15 minutes under anaerobic conditions but 33 minutes when grown aerobically.

No.10 (04 70 01 - In vitro studies in ion movement in human jejunal mucosa)

Net ion movements across small specimens of human jejunal mucosa using the short circuit current (Isc) technique have been measured. Specimens from seven patients with acute cholera demonstrate a depression in Isc of 30% (range 11-56%) when compared with tissue obtained from the same cases in convalescence. Many of the mucosa samples when exposed to cholera culture filtrate (NIH Lot No.001) respond, with a characteristic time lag similar to rabbit ileum, with a rise in Isc. This response in rabbits has shown to be due to anion hypersecretion.

Thus, the change in Isc in human jejunal mucosa infected with cholera is opposite to that produced by a culture filtrate toxin on the same tissue. Whereas anion hypersection has been shown to be the cause of increased Isc in the toxin challenged rabbit ileal mucosa, these results are inconsistent with such a mechanism in natural human cholera but are consistent with a decreased sodium absorption as the primary aberration.

No.11 (04 70 05 - Osmolar regulation of intestinal fluid)

Osmolar regulation has been studied in loops of dog jejunum into which are put different concentrations of mannitol before and after treatment with NIH toxin.

Osmolarity in the loop approaches that of plasma more slowly after toxin treatment, apparently because absorption of mannitol and unidirectional outflux of Na are both depressed. In some cases, influx of Na is also increased after toxin.

No.12 (07 70 02 - Iron absorption in Sylhet Tea Garden)

Ferrous iron absorption has been studied in twenty patients selected from a series of over two hundred hypochromic anemias in Sylhet Tea Garden workers. Patients who responded to oral iron satisfactorily exhibited the expected avid uptake of Fe^{59} tracer, and normal controls showed a predicted lower absorption. Three of six anemic patients who did not show a reticulocytosis following oral iron but did increase hematocrit in response to parenteral iron demonstrated very low absorption of tracer. Though these cases had no obvious clinical manifestations of malabsorption xylose absorption was markedly depressed. Thus, it is suggested that malabsorption of dietary iron may play a role in the anemia of these patients.

No.13 (CI/P31 - Age specific electrolyte composition of cholera stool)

The electrolyte content in the stool of children with cholera has been shown to be substantially different from that found in adults with cholera. The development of an effective oral solution for use in children depends on an understanding of when a child becomes an adult with respect to the electrolyte composition of his stool. Thirty seven patients were studied. The sodium and potassium concentrations of cholera stool change gradually with increasing age, demonstrating no abrupt rise at puberty. The sodium and potassium concentrations are also related to stooling rate, i.e., the lower the stooling rate the more pediatric-like the stool is. Studies by Mahalabis have demonstrated that the electrolyte concentration in the small intestine up to the ileocecal valve of the pediatric patient is similar to the content of adult stool. Therefore, the colon makes the pediatric stool electrolyte composition different from that in the adult.

No. 14 (04 70 10 Studies on body composition in cholera patients)

Body composition has been studied in a total of 33 adults and 39 boys. Whole body density can be done only in males. Total body water in adults was measured with tritiated water also. Results of the adult series have been written up and submitted for publication. Results of studies in children are not yet analyzed thoroughly, but the striking finding in all subjects is the very low value for fat, averaging 10% in adult males, less than that in boys and only 16% in females.

The organization of the Physiology Division is shown in the Administrative Report to the Directing Council.

Miss Torrance is the Superintendent of the Hospital Branch and is responsible for hospital and nursing care for the patients, for the operation of the laundry, pharmacy, kitchen and for the production in intravenous fluids. The ward still lacks hot water, adequate facilities for storage and preparation of food, for storage of hospital equipment and space for patients during an epidemic. The low mortality rate and the growing acceptance of PSCRL ward by the community are tributes to the Superintendent's capacity for effective work under adverse circumstances.

During FY69 the intravenous fluid unit produced 24,779 liters of intravenous fluids; 22,480 were consumed. This narrow reserve was maintained during the epidemic in Matlab by the use of oral therapy.

The staff physicians under Dr. Alam have also contributed to the excellent record of the ward. The staff has been decreased by the departure of three; Drs. Saha, Molla and Rahman for higher education.

Those directly responsible for the investigations include Drs. Aziz, Ally, Cash, Barry, Nalin, Curlin, Rahaman and Rohde. Dr. Barry is on Sabbatical leave from the University of Maryland.

The animal house has been renovated and expanded to include dog runs, sheep shed and locker room. The caging for breeding stock is still not adequate and the diets, except for rats, are not satisfactory. For these reasons we have imported rabbits from the U.K. and must now establish new colonies of guinea pigs and rabbits. The supply of local dogs is satisfactory.

The statistical tables of library acquisitions and library services indicate increases in periodical resources. With an increasing number of research investigators reading a larger number of periodicals the circulation figures accelerated sharply. Due to the availability of the Xerox 914 Copier, copy services have shown an appreciable increase over the previous year. The loan of bound journals decreased due to this facility.

Twenty new periodicals were subscribed during the fiscal year and three periodicals were discontinued.

The Librarian attended the Congress held in Amsterdam, May 5-9, 1969, as the representative of the PSCRL and Pakistan. Attendance at this conference was of great assistance in evaluating PSCRL library operations and provided a treasure of contacts with foreign librarians who now take an active interest in the library of the PSCRL.

Twenty new book cases and three filing cabinets were accommodated in the library in January 1969. The limited area is now over-furnished and there is no room for incoming material. The library is desperately in need of more space and an air-conditioned facility for storage of back issues of useful journals.

Table I Library Acquisitions

	FY65	FY66	FY67	FY68	FY69
Books added	158	856	662	654	368
Bound journals added	220	348	925	301	553
Reprints cumulative total	-	-	-	2520	3320
Periodicals subscribed (cumulative total)	80	146	158	196	216

Table II Library Services

		FY68	FY69
Current periodicals circulated		5,835	15,567
Reprints circulated		4,681	6,837
Photocopies provided:	PSCRL	4,132	12,589
	NLM	3,691	1,783
	WHO	28	20
Bound journals loaned		1,940	5,146
Books referred to in Library		11,904	10,657

The Clinical Pathology Unit has suffered from chronic neglect. Mrs. Clarissa Bart, a highly capable laboratory supervisor, is rapidly rehabilitating the unit.

The Chemistry Branch has acquired a second scintillation counter and has two flame photometers on order. The personnel has been reduced by the departure of the for graduate study or more lucrative employment. This depletion is now serious.

REPRESENTATION

FY69

MANUSCRIPTS REPORT
FY69

Constance G. Miccio

One hundred fifty-one separate manuscripts generated by the professional staff were produced by the clerical staff of the PSCRL during the reporting period. Of these, 84 were published or accepted in full or abstract, another 32 were presented at meetings or accepted, and 32 were "in process" (manuscripts submitted to publishers who had not yet replied and material in various stages of draft) by June 30, 1969. Included in the above total are eight manuscripts withdrawn for various reasons after submittal, material rejected by publishers and then in process of revision, and material accepted in FY69 for publication or presentation in FY70.

Not included in the above total are two bound volumes (totalling approximately 500 pages) of the Proceedings of the Directing Council and the Proceedings of the Technical Committee - reports produced for the annual meetings in Dacca and then published by the PSCRL for a limited distribution. Material for the FY68 edition, produced in FY69, introduced new pages (Tables of Contents and Honors and Awards) and pagination. Material for the reports arrive from each section of the PSCRL and from outside the organization as well, often not in accordance with any particular format or page size suggestive of one publishable standard. An attempt was made in the FY68 editions to arrange the material in some coordinated manner, re-produce or re-format other material that disagreed markedly from that produced by PSCRL, and thus hope that this end-product would altogether be more representational of the fine work ongoing at the PSCRL as well as serve end-users to a larger degree. Enlisted to assist in the production of the books were administrative assistants, secretaries, and typists throughout the organization - none of whom had had any particular direction or specific training or indoctrination in publishing of material of this kind, although most had contributed the initial material. Some political

turbulance and a natural disaster in Dacca disrupted production last year of the final editions, but the product itself was more than adequate. Production of the FY69 editions is ahead of schedule and being produced by the same staff of approximately 11 persons who contributed last year.

Also not included in the above total are various combined administrative and technical reports that require coordinating, editing and production, such as the Annual Report to the Director of the U.S. National Institutes of Health.

Largely as an outcome of various problems encountered during preparation of the Proceedings for FY68 and general dissatisfaction with the quality of products being submitted to publishers - not to be confused with content - considerable attention has been given during the past year to the matter of manuscripts. Modern practices, such as using guide sheets, have been introduced, as have a few sophisticated products and processes since installation of a Rank Xerox copying machine (which had just become operational shortly before production of last year's editions). Plastic covers and "Sno-pake" have been in use for some time and typing of "masters" from which Xerox copies may be reproduced is now standard practice. Equipment has been shifted to facilitate uniformity of size of type and procedures for handling this material have been standardized insofar as possible with such wide range of end-users, largely publishers. These few innovations, however, have resulted in major time savings for staff professionals and the increased speed of output in the production of finished copy is pronounced - one manuscript may be divided among several typists and two-thirds, if not more, of the editing and re-proofreading of re-typed material is no longer necessary or is in the nature of major revisions. Five typists are specializing in manuscripting at the present time in addition to producing general non-statistical, non-technical material.

Most of the supplies have had to be programmed for receipt by surface from the U.S. which has afforded considerable cost reduction (example: typewriter ribbons, $.15 shipped from U.S. vs. $3.00 local market price).

Distribution of reprints or reproduction of staff-produced, published material have, of necessity due to copyright factors, been handled in the Director's office. A survey was made of these operations inclusive of a review of mailing costs and practices. Introduction to certain sensitive aspects of copyrights, explanation of some general geographical elements, training in the use of unique mailing facilities available, and correction of duplications and errors in distribution lists have resulted in greater understanding by the staff charged with this work. This has also facilitated a more equitable distribution of reprints to authors on the staff as well as to requestors and promoted guidelines for assembly of "kits" of recent reprints which are more representative of the work of all of the professionals on the staff (for handing out to official, professional visitors or requestors by mail).

Another equally significant benefit has been that no manuscripts have "gone astray" due to mistaken addressing, nor been damaged, for well over one year; as a matter of fact, material that has been returned has been received. Possibly location on local market of better quality mailing envelopes for manuscripts has contributed to the latter fact as has correction of the PSCRL return address on stationery, envelopes, reprint forms, and manuscripts.

Despite the great number of reprints being mailed regularly - which accelerates year by year as more material is accepted for publication - the cost of such bulk mailing has not previously been reviewed as an expense line item. A particularly significant benefit of a survey having been made and some guidance given to the staff is a savings in postage costs. Assembly of this detail may be useful for budget purposes.

The survey revealed that 75% of the mailing is to the U.S.A. at the present time using the upgraded distribution list. Formerly all of this material had been sent via international mail despite significantly higher postage. It is recommended that pre-affixed indicia envelopes be obtained from U.S. sources for use on manuscripts and reprints only since this would afford still cheaper postage at bulk rates (this is justifiable since this material is a product of 87% USG funded programs) which would enable reductions to be made in addition to the following.

A significant savings has been realized for the past year for reprints mailing. The average weight of envelopes containing a reprint is 3 oz. The mailing of 3 oz. envelope through official channels to the U.S. requires, at the present time, affixing of $.06 postage vs. mailing of same envelopes through open mail at per unit cost of $.50. A fairly standard stock of reprints per published article which will be mailed is 200. Until FY69 distributions totalled approximately 15,300 units. It is predictable that 57 orders will have been placed for products of FY69 which may result in mailing of 11,400 units, 7,590 to U.S., at a savings of $3,762.00.

Some savings have been realized in the mailing of manuscripts. The mailing of an 8 oz. manuscript through official channels to the U.S. requires, at the present time, affixing of $.80 postage vs. mailing of same manuscript through open mail at a unit cost of $3.28. Fifteen manuscripts were sent to U.S. publishers in FY69 at a savings of $37.20, although this figures does not allow for material that may have been handcarried. It follows that greater emphasis on reaching this media would result in proportionate savings in postage costs.

Better recordkeeping, greater accuracy and speed, expanded understanding of and identification with ongoing studies and a mounting pride in quality of workmanship by PSCRL clerical staff is a small but significant contribution to programs. This report

would not be complete if the work of clerical personnel in Finance Management Branch and Epidemiology Division were overlooked in emphasizing contributions made by specialists previously mentioned. Several large financial statements typed on stencils, which constitute the financial and budget reports that are issued for the annual meetings and later provided for inclusion in the Proceedings, have been prepared in the FMB by typists assigned to that section after this material is coordinated by the Director's office to key margins and paper size to the remainder of the material. The secretary to the head of Epidemiology has, despite being part-time, produced most of the manuscripts submitted by that section either in draft or final.

Appended are lists of manuscripts and presentations for FY69.

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FY69

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(Detailed provided if available)

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PRESENTATIONS AT MEETINGS
FY69

SYMPOSIUM ON MEDICINE, INSTITUTE OF POSTGRADUATE MEDICINE, DACCA, EAST PAKISTAN, June 28-29, 1968.

Northrup, R.S.: Advances in cholera pathophysiology and therapy. (Abstract published by the Institute, pp. 74-85, February 1969.)

7TH ANNUAL MEDICAL SYMPOSIUM, JINNAH POSTGRADUATE MEDICAL CENTER, KARACHI, WEST PAKISTAN, August 12-17, 1968. (Abstracts published by Symposium.)

Aziz, K.M.S. and Mohsin, A.K.M.: Highly potent Vibrio cholerae exotoxin, p. 43.

Bhattacharjee, A.K. and Mosley, W.H.: Studies on the nature of antibodies in cholera, p. 41.

Cash, R.A., Mollah, M., Haque, Z., Toha, M.M., and Nalin, D.R.: Acetate as a base precursor in the correction of acidosis secondary to diarrhea and its use in intravenous fluid, p. 42.

Khan, M. and Mosley, W.H.: Incubation period and hours of onset of cholera, p. 43.

Nalin, D.R., Islam, R., Cash, R.A., Mollah, M., and Phillips, R.A.: Clinical trial of oral solution for treatment of cholera, p. 42.

Northrup, R.S.: Immunoglobulins in the intestine and stool of cholera patients, p. 47.

Rahaman, M.M. and Rahman, G.S.: Follow-up of severely malnourished children admitted to the Pakistan-SEATO Cholera Research Laboratory between 1962 and 1967, p. 39.

Rahaman, M.M. and Rahman, W.: Electrolyte composition of diarrheal stool in children - some thoughts on proper replacement therapy, p. 45.

Rahman, R., Kibriya, G., and Huq, I.: Intestinal bacterial flora in normal adults of East Pakistan, p. 46.

8TH INTERNATIONAL CONGRESSES ON TROPICAL MEDICINE AND MALARIA, TEHERAN, IRAN, September 7-15, 1968.
(Some papers appeared in Abstracts and Reviews issued by the Congresses, all papers were to be published in full in a supplement. No detail available.)

Aziz, K.M.S. and Mohsin, A.K.M.: Cholera toxin from culture filtrates of human cholera stool, Abstracts and Reviews, pp. 487-488.

McCormack, W.M., Chowdhury, A.M., Jahangir, N., Ahmed, A.B.F., and Mosley, W.H.: Tetracycline prophylaxis in the families of cholera patients, Abstracts and Reviews, pp. 518-519.

Mosley, W.H.: Cholera vaccine field trials. (This was substituted on the program for a paper originally scheduled to be given by Dr. Jesus Azurin of Manila, Philippines. It did not appear in Abstracts and Reviews.)

Mosley, W.H.: Recent developments in cholera immunizations, Abstracts and Reviews, pp. 1067-1068.

Mosley, W.H.: The surveillance of cholera. (This was given before the Intercongressional Plenary Session No. 4, entitled 'Dynamics, Surveillance and Control of Infections and it did not appear in Abstracts and Reviews.)

Nalin, D.R., Cash, R.A., Islam, R., Mollah, A.M., and Phillips, R.A.: Clinical trial of an oral solution for the treatment of cholera, Abstracts and Reviews, p. 502.

Nalin, D.R., Cash, R.A. and Phillips, R.A.: Acetate as a base precursor in the correction of acidosis secondary to diarrhea and its use in intravenous fluids, Abstracts and Reviews, p. 501.

Northrup, R.S., Kinzie, J.L., McCormack, W.M., and Phillips, R.A.: Tetracycline lavage used to clarify the recovery process in cholera, Abstracts and Reviews, pp. 489-490.

Rahaman, M.M., Rahman, W., Hare, W.K., Hare, R., and Phillips, R.A.: Electrolyte and fluid balance in pediatric cholera, Abstracts and Reviews, p. 490.

CHOLERA WORKSHOP, an informal meeting concerned with pathophysiology and immunology aspects of cholera. The meeting was sponsored jointly by the NIH Cholera Advisory Committee and the U.S.-Japan Cooperative Medical Science Program committee and was held at the National Institutes of Health, Bethesda, Maryland, U.S.A., October 2-3, 1968. The workshop was attended by Drs. Robert A. Phillips and W. Henry Mosley. The reports given were informal and neither proceedings nor abstracts were published.

NIH CHOLERA ADVISORY COMMITTEE meeting, National Institutes of Health, Bethesda, Maryland, U.S.A., October 4-5, 1968. The meeting was chaired by Drs. John R. Seal and was attended by Drs. Robert A. Phillips, W. Kendrick Hare, W. Henry Mosley and K.M.S. Aziz. A copy of agenda detail was not received and abstracts were not published.

UNIVERSITY OF WEST VIRGINIA, DEPARTMENT OF PHYSIOLOGY AND BIOPHYSICS, MORGANTOWN, WEST VIRGINIA, U.S.A., October 10, 1968 (lecture).

Aziz, K. M.S.: Cholera toxin and its effects.

U.S.-JAPAN CONFERENCE, Cholera Symposium, Unzen, Nagasaki, Japan, November 4-6, 1968. Afternoon session, November 5, 1968 on Epidemiology, phage, antigenic structure of Vibrio cholerae, and diagnostic techniques of cholera. (No detail available.)

Mosley, W.H.: Recent developments in cholera immunization.

5TH CONFERENCE OF THE INTERNATIONAL EPIDEMIOLOGICAL ASSOCIATION, August 1968, Primosten, Yugoslavia. Conference organized by the Union of Medical Societies of Yugoslavia, Belgrade, Yugoslavia. (To be published in full by the Society.)

Mosley, W.H. and Langmuir, A.D.: The evaluation of cholera vaccine in an endemic area.

CENTO SYMPOSIUM ON DEMOGRAPHIC STATISTICS, KARACHI, WEST PAKISTAN, November 5-12, 1968. (Symposium organized by CENTO secretariat in Ankara, Turkey; program handled by Central Statistical Office ISMCH Society, Karachi. No detail available.)

Ahmad, S. and Gesche, M.C.: Preliminary report of a maternal mortality study conducted at Matlab (Comilla District), East Pakistan.

Aziz, K.M.A., Chowdhury, A.K.M.A., and Mosley, W.H.: Population studies in rural East Pakistan.

Chowdhury, A.K.M.A. and Aziz, K.M.A.: Possible limitations of the family planning program on population growth of East Pakistan.

10TH ALL-PAKISTAN MEDICAL CONFERENCE (Pakistan Medical Association), DACCA, EAST PAKISTAN, November 14-17, 1968. (All abstracts published in "Souvenir" brochure, unpaginized.)

Aziz, K.M.S., Mohsin, A.K.M., Hare, W.K., and Phillips, R.A.: Studies on cholera toxin with animal models including rats.

Cash, R.A.: Recent development in the pathophysiology of cholera.

Islam, M.R., Mollah, A.M., Saha, P.S.K.: Intravenous therapy of cholera.

Monsur, K.A., Northrup, R.S., Hirschhorn, N., and Rahman, M.A.: Preliminary studies of phage for the treatment of cholera.

Mosley, W.H., Fahimuddin, M., Aziz, K.M.A.: Cholera vaccine studies in rural East Pakistan.

Nalin, D.R., Cash, R.A., Islam, R., Mollah, A.M., and Phillips, R.A.: Oral therapy of cholera.

Northrup, R.S.: Using of antibiotics in cholera including clinical trial of Ampicillin.

Rahman, M.: Experiences in treating cholera in a rural hospital.

Rahaman, M.M. and Rahman, W.: Some problems of cholera therapy of children.

INTERNATIONAL CONGRESS OF PEDIATRICS, MEXICO CITY, MEXICO, December 1-7, 1868. (No detail available)

Rahaman, M.M., Rahman, W., Hare, W.K., and Hare, R.: Electrolyte and fluid balance in pediatric cholera.

4TH DIVISION MEDICAL CONFERENCE (Pakistan Medical Association), KHULNA, EAST PAKISTAN, February 2-4, 1969. (Papers abstracted in Souvenir program, unpaginized.)

Alam, A.K.M.J.: The practical treatment of cholera.

Music, S.: Recent advances in epidemiology and pathophysiology of cholera.

Nalin, D.R. and Cash, R.A.: Complications seen in cholera therapy and recent developments in therapy.

Rahaman, M.M.: Problem of diarrhea and malnutrition in children.

NIH CHOLERA ADVISORY COMMITTEE meeting, National Institutes of Health, Bethesda, Maryland, U.S.A., March 21-22, 1969. The meeting was chaired by Dr. John R. Seal and attended by Drs. Robert A. Phillips, W. Kendrick Hare, W. Henry Mosley, George T. Curlin, and Stanley Music. The agenda included the following presentations.

Immunology and Epidemiology

Molla, M., Hirschhorn, N., Gangarosa, E., Mosley, W.H., and Martin, W.: Detection of the El Tor and classical biotypes of Vibrio cholerae in convalescent cholera patients by purgation. Presented by Dr. Gangarosa.

Martin, W.R. and Burrows: Chemical properties of ileal loop toxin. Presented by Dr. Martin.

Curlin, G.T., Subong, A., and Craig, J.P.: Antitoxic immunity in experimental canine cholera. Presented by Dr. Curlin.

Mosley, W.H.: The role of immunity in cholera. Presented by Dr. Mosley.

Pathophysiology

Kinzie, J.L., Taylor, J.O., Wallace, C.K., and Iber, F.L.: Cation flux studies in clinical cholera in experimental cholera models.

EPIDEMIOLOGIC INTELLIGENCE SERVICE (EIS) CONFERENCE, ATLANTA, GEORGIA, U.S.A., April 12-20, 1969. (All abstracts published).

Bart, K.J.: Preliminary observations on the epidemiology of El Tor cholera in Chittagong (East Pakistan).

Woodward, W.E.: The spectrum of cholera in rural East Pakistan.

50TH ANNUAL SESSION OF THE AMERICAN COLLEGE OF PHYSICIANS, PHILADELPHIA, PENNSYLVANIA, U.S.A., April 20-25, 1969.

Nalin, D.R., Cash, R.A., and Phillips, R.A.: A field trial of oral therapy in cholera. Abstracted in Medical News of the College, 2: No. 1, November 1, 1969.

3RD INTERNATIONAL CONGRESS ON MEDICAL LIBRARIANSHIP, AMSTERDAM, HOLLAND, May 5-9, 1969.

Molla, P.: Academic medical libraries in Pakistan. (This will be abstracted in Excerpta by the Medical Foundation, Amsterdam.)

Molla, P.: An international biomedical library in Dacca and the use of worldwide medical information services. (This will be abstracted also by the Foundation. Permission has been obtained by the East Pakistan Library Association to publish the paper in full in the Eastern Librarian.)

ROSS INSTITUTE OF TROPICAL HYGIENE, PAKISTAN BRANCH, BALISERA MEDICAL DEPARTMENT, KALIGHAT, SOUTH SYLHET, EAST PAKISTAN, May 5-11, 1969. (Lectures, unabstracted.)

Bart, K.J.: Child welfare and well baby care.

Bart, K.J.: Malnutrition.

Cash, R.A.: Hypertension.

Mosley, W.H.: Communicable diseases.

Mosley, W.H.: Vital statistics.

Northrup, R.S.: Bowel infection.

WASHINGTON UNIVERSITY ANNUAL SCIENTIFIC PROGRAM, ALUMNI ASSOCIATION OF THE SCHOOL OF MEDICINE, ST. LOUIS, MISSOURI, U.S.A., June 5-6, 1969. (Lecture, no information available.)

Phillips, R.A.: International medical research on cholera.

PRESENTATIONS ACCEPTED IN FY69
FOR PRESENTATION IN FY70
(Detailed provided if available)

8TH ANNUAL MEDICAL SYMPOSIUM, JINNAH POSTGRADUATE MEDICAL CENTER, KARACHI, WEST PAKISTAN, August 8-9, 1969.
(Papers submitted in June. Five additional papers were not accepted. It is practice of JPMC to abstract papers.)

Ahmed, A., Mosley, W.H., and Aziz, K.M.A.: Serological evidence for the identity of the vascular permeability factor and ileal loop toxin of Vibrio cholerae.

Ahmed, S. and Gesche, M.C.: Maternal mortality study in the Matlab area of rural East Pakistan.

Aziz, K.M.S.: Studies on bacteriophage in relation to cholera.

Bart, K.J. and Khan, M.U.: Isolation of Vibrio cholerae from night soil during epidemics of classical and El Tor cholera.

Barui, R.K., McCormack, W.M., and Mosley, W.H.: A study of purging for the detection of inapparent infections in the families of cholera patients.

Chowdhury, A.K.M.A., Aziz, K.M.A., and Mosley, W.H.: Fetal and neonatal mortality in rural East Pakistan.

Huq, M.I., Dey, C.R., and Huq, A.: Sulfa sensitivity in the differentiation of vibrio coma and vibrio El Tor.

Rahaman, M.M., Rohde, J.E., Molla, A.M., and Rahman, W.: Total body water, blood volume, bromide space and body fat in adult East Pakistanis.

Rahman, A.S.M.M.: Clinical comparison between furazolidone and tetracycline in the different doses in the treatment of cholera.

4TH SINGAPORE-MALAYSIA CONGRESS OF MEDICINE, SINGAPORE, SINGAPORE, August 14-17, 1969. (Papers submitted in June. Two additional papers were submitted and later withdrawn. Detail not available.)

Cash, R.A. and Nalin, D.R.: Oral cholera therapy.

Nalin, D.R., Cash, R.A., Reller, B., Rochat, R., and Haque, Z.: Treatment of cholera with fluid replacement and an oral adsorbing agent.

ROUND TABLE CONFERENCE ON VACCINES, INTERNATIONAL ASSOCIATION OF MICROBIOLOGICAL SOCIETIES, BERNE, SWITZERLAND, OCTOBER 9-11, 1969. (Permanent Section of Microbiological Standardization, Panel on Salmonella, Shigella and Vibrio Cholerae. Paper submitted in April and abstracted in "Programme and Abstracts" by the Association.)

Mosley, W.H., and Feeley, J.C.: The relationship of cholera vaccine effectiveness to serological responses in humans and to the mouse protection test.

PROCEEDINGS OF THE MEDICAL RESEARCH SOCIETY, THE ROYAL FREE HOSPITAL, LONDON, ENGLAND, October 17, 1969. (Presented by Dr. Love and abstracted by the Society.)

Love, A.H.G., Rohde, J.E., and Veall, N.: Sodium transport in the human intestine, p.3.

REPORT OF THE TECHNICAL COMMITTEE

FY69

REPORT OF THE
SEVENTH MEETING OF THE TECHNICAL COMMITTEE
December 1-3, 1969

The Technical Committee met in joint session with the Directing Council, three members of the Clinical Investigation Committee and four Consultants to the PSCRL.

In attendance at the meetings were the following:

Members of the Technical Committee

Dr. Colin MacLeod
New York University School of Medicine, U.S.A.
Chairman of the Technical Committee
Government of the United States of America

Dr. Ziaur Rahman
Deputy Director General, Health and
Deputy Secretary, Health Division, Islamabad, West Pakistan
Government of Pakistan

Sir James W. Howie
Director, Public Health Laboratory Service, London, England
Government of the United Kingdom

Members-designate of and representatives to the Directing Council

Dr. Robert Cruickshank
Professor Emeritus, University of Edinburgh, Scotland
Chairman of the Directing Council
Delegate of the United Kingdom

Colonel M. M. Haque
Secretary, Health Department, and
Director, Health Services
Government of East Pakistan, Dacca, East Pakistan
Delegate of the Government of Pakistan

Dr. Jean F. Rogier
Chief Public Health Advisor, East Pakistan
U.S. Agency for International Development in Pakistan, Dacca
Delegate of the Government of the United States of America

Dr. John R. Seal
Chairman, NIH Cholera Advisory Committee and
Scientific Director, NIAID
National Institutes of Health
U.S. Department of Health, Education and Welfare
Public Health Service, Bethesda, Maryland, U.S.A.
Delegate of the Director of the
National Institutes of Health

Members of the Clinical Investigation Committee

Dr. Nazir Ahmad
Joint Secretary, Health
Government of West Pakistan, Lahore, West Pakistan

Dr. Graham Bull
Director, Medical Research Council
Clinical Research Center, London, England
Government of the United Kingdom

Dr. A. K. Shamsuddin Ahmed
Retired former Director
Institute of Chest Diseases and Chest Hospital
Government of East Pakistan, Dacca, East Pakistan

Consultants

Dr. Alexander Langmuir
Chief, Epidemiology Program
National Communicable Diseases Center
U.S. Department of Health, Education and Welfare
Public Health Service
Atlanta, Georgia, U.S.A.

Dr. T. Hastings Wilson
Department of Physiology
Harvard University Medical School
Boston, Massachusetts, U.S.A.

Dr. Alan Skyring
A. W. Morrow Department of Gastroenterology
Royal Prince Alfred Hospital
Camperdown, Australia

Professor W. E. van Heyningen
Master, St. Cross College
University of Oxford, Oxon, England

Observers

Dr. K. A. Monsur
Director, Institute of Public Health
Government of East Pakistan, Dacca, East Pakistan

Colonel J. K. Fleming
Chief Medical Officer (posted by Australia)
South-East Asia Treaty Organization, Bangkok, Thailand

Dr. A. K. M. Abdul Wahed
Former Director, Health Services
Government of East Pakistan
Dacca, East Pakistan

Dr. Paul Hamilton
Alfred I. du Pont Institute
Wilmington, Delaware, U.S.A.

The Technical Committee notes with deep regret the death of Mr. David A. Wraight on November 4, 1969. Mr. Wraight served for a number of years on the Directing Council representing the Secretary General of SEATO. His concern for the welfare of the Laboratory and his warm friendship are greatly missed.

The Technical Committee appreciates the assistance given by all these men and their interest in the work of the Laboratory. Our work was made efficient and pleasant because of the excellent cooperation of the Director and all of his staff.

We wish once again to compliment highly the Division heads and their associates on their oral and written presentations of work completed during the past year and their succinct protocols for future studies. This has been accomplished in the face of a heavy clinical load because of a severe epidemic of cholera for the second successive year.

We have now had a second experience of a joint meeting of the Technical Committee, the Directing Council and consultants to the Laboratory. These experiences convince us that a joint meeting is desirable for the future and we so recommend.

During the present meeting the Technical Committee was in session for three full days, with an additional day intervening before the meeting of the Directing Council. We recommend that this schedule be followed in the future.

Our meetings for the past few years have been held during the height of the cholera season. We believe that this places an unnecessary burden on the staff of the Laboratory because of their other urgent duties. We recommend, therefore, that the next meeting of the Technical Committee and the Directing Council be held during the last week of October 1970, with the assurance that the annual audit can be completed sufficiently in advance of that time.

ORGANIZATION

The Technical Committee wishes to note with approval the reorganization of the Laboratory into four divisions: Administrative, Maintenance, Epidemiology and Physiology.

ADMINISTRATION

It has been brought to the Committee's attention that considerable uneasiness exists among some members of the

professional staff concerning the current and projected programs of the Laboratory. The Committee is deeply concerned over these developments. While we have no specific recommendations to make we urge that these questions be given immediate attention as matters of the highest priority.

PERSONNEL

On June 30, 1970 Dr. W. Kendrick Hare and Dr. (Mrs.) Ruth Hare will leave the Laboratory, to which they have given devoted service of the highest quality since September 1966. In expressing its grateful thanks and good wishes to Kendrick and Ruth Hare, the Technical Committee notes this change as one of critical importance to the life and work of the Laboratory. Exact replacement of the Hares will not be possible.

Five posts were judged to be of particular importance and the Technical Committee recommends that they should be filled as fast as possible in order to continue the ongoing work of the Laboratory, to close gaps that have appeared and to meet new commitments set out in the protocols presented to the Committee. The numbering of the posts does not necessarily indicate priority ranking.

Post No.1 is for a biochemist (or physiologist) with a special interest in problems of electrolyte transport. The person appointed would be responsible for quality control of all chemical procedures undertaken.

Post No.2. It remains a serious deficiency that the Laboratory lacks an experienced bacteriologist with a good record of research. It is self-evident that a major requirement is adequate quality control of the routine and supporting work of the Laboratory. There is need also for formulation of research projects aimed at gaining a better understanding of the fundamental biology of Vibrio cholerae and at seeking markers which could make possible a typing scheme sufficiently detailed and reproducible to be of value in tracing the origin and spread of individual outbreaks of cholera.

Post No.3. An immunologist, preferably with an interest in the chemical side of the subject, is needed for work set out in both the epidemiology and the physiology programmes. The services of an immunologist are required in field and experimental work on the toxin and on the different possible forms and modes of use of toxoids and vaccines. Also, further chemical investigation is required to define the nature and specificity of various antibodies and neutralising substances found within the intestinal tract and elsewhere.

Post No.4. For the animal breeding programme, which is central to all the experimental work of the laboratory and to many of the epidemiological studies, it is essential to recruit the services of a man with a special interest in and experience of animal husbandry. The person appointed might have a veterinary training but this would not be regarded as essential. The need is to find someone with real skill and capacity in the breeding and maintenance of animals and a sincere interest in their welfare.

(Note: The Committee regards it as not impossible that, for a time at least, the posts numbered 2, 3, and 4 might be filled by two persons because it is possible to find, for example, veterinary graduates with an interest in animal breeding and maintenance as well as in either bacteriology or immunology. Similarly, some bacteriologists are competent immunologists and vice versa. In the end, however, all three posts should be filled.)

Post No.5. This is for an epidemiologist. A recommendation for the creation and filling of this post has appeared in three previous reports of the Technical Committee (1966, page 7; 1967, page 13; and 1968, page 9). The arguments previously advanced remain valid and there is now both need and opportunity for further epidemiological studies, particularly demographic studies. Taken along with the extended trials of vaccines and new toxoid preparations, these indicate clearly an increased load of work upon epidemiology. For this reason the Technical Committee now recommends the filling of this post as a matter of urgency.

FACILITIES

During the past year much has been accomplished in improving the Laboratory's physical facilities through renovation of existing space and construction of the new maintenance and supply building. The amount of new laboratory furniture that has been built and installed is impressive, as is the quality of the workmanship.

It is particularly gratifying that most of the problems encountered for so many years because of an inadequate electric power supply will be relieved in the very near future with completion of the new substation and its connections to the various buildings and internal wiring.

The Technical Committee applauds, likewise, the complete renovation of the animal house, which now appears adequate for the needs of the immediate future with respect to space and caging for animals, experimental rooms, washing facilities, storage and the like. We hope that an adequate breeding colony for rabbits can now be operated successfully and thus relieve the large expenditures presently necessary in importing rabbits from England by air. It seems not unlikely, however, that success in rabbit breeding may have to await the recruitment of someone highly skilled in animal husbandry or in veterinary medicine.

Despite these great improvements for which much credit is due the staff, especially Mr. Mark Tucker, the Technical Committee is concerned that renovation and extension of the ward space has lagged behind. It remains true, to the great credit of all concerned, that the ward provides exemplary care for cholera patients. This is carried out in extremely crowded space with grossly inadequate supporting services. While patient care has been excellent we wish to note that the ward is the face the Laboratory presents to the Pakistani public and to visitors from abroad. Its inadequacies, moreover, place an unnecessarily heavy burden on Miss Torrance and her staff. This has been particularly apparent during the very large epidemic of last year as well as during the present epidemic which already promises to equal or exceed the previous one. Nor does it seem likely that the load on

the ward will diminish in the future. It is almost sure, rather, that it will continue to increase, limited only by the availability of facilities to transport patients to the Laboratory.

For these reasons the Technical Committee urges that vigorous action be started once again to expand the ward space through construction of the previously proposed one-storey building adjacent to it and extending out to the road, and through improvement of the facilities for care of convalescent patients.

Expansion of the ward area will permit adequate separation of adult male and female patients and provide enough space for children so that there is room for the staff to move freely between the beds and stretchers in carrying out treatment procedures.

A new laundry shed has been built which will accommodate the commercial size washer, extractor and dryer which are on order. The new steam generating plant to be installed will provide hot water for laundry and kitchen and for general ward use. This will be a major improvement.

There is also an immediate need to renovate the kitchen and to provide refrigerated storage for food.

A perennial difficulty at the Laboratory has been the water supply. The single water tower and single pump are inadequate. If power fails, a frequent occurrence in the past, or if the pump breaks down, the water supply will last for not more than two hours. While this is a serious matter for the various laboratories, it is much more so with respect to care of patients.

We concur fully with the recommendations of the Director and his staff that a new and larger water tower be built over its own new well and with a separate pump.

There seems to be little possibility, because of lack of space, that much can be done in the near future to relieve the congestion in the library area so that it can be made adequate for the constantly growing needs of the Laboratory.

Effective relief of the library problems, as well as of the extreme crowding in the laboratories, is dependent on construction of the proposed new four-storey laboratory building to be connected to the present building. Plans for this new building have been drawn and approved by the East Pakistan government, but construction funds have not been appropriated. With expansion of the Laboratory's activities into demography, family planning and prevention of fetal wastage, and into nutritional studies, the need for the new building becomes critical in order to house the expanding staff and the necessary equipment. The Technical Committee recommends strongly that steps be taken to obtain the funds needed for construction of the new building. It should be noted that if this building can be built soon the need for the additional ward space will be taken care of. However, we do not believe that the future prospects of the new laboratory building should be allowed to diminish the efforts to relieve the urgent present need for expanded and improved ward facilities.

PHYSIOLOGY DIVISION

Introduction.

Cholera is primarily a disease of poor people in poor communities, most of which are remote from transport or other amenities. Untreated, the fatality rate varies from sixty to ninety percent and death may occur as early as six hours from onset of the disease. These facts place very severe constraints on the practicability of treatment.

Based on current knowledge of the disturbed physiology in the disease, two regimes of therapy have been developed which are highly effective in particular favoured environments but which in field conditions leave much to be desired.

The first of these is the intravenous administration of a mixture of water and electrolytes developed by Dr. Phillips and his teams both here and in Taipei. Even if applied to moribund patients it leads to recovery and by its use the fatality rate could be reduced to virtually zero if it were possible to bring the patient to the treatment or the treatment to the patient in time. Unfortunately, there is no prospect of doing either except in special circumstances such as cities. An additional factor that almost rules it out for field use is cost, which in East Pakistan amounts to five month's wages of the average worker.

The second, an oral regime, is much cheaper (two days wages) but at the present stage of development this regime is incapable of saving the most severely ill patients. It might be possible to deploy it at village level; if so, this would probably reduce the fatality to approximately five to ten percent, a figure which is still unacceptably high.

Therefore, in the present state of efficacy of vaccines there is still a very great need for further developments in treatment. Moreover, even if a vaccine was developed which gave 100 percent protection to those receiving it there would still be a need for treatment, for experience in East Pakistan with smallpox vaccination has shown that large numbers of the population remain unvaccinated and are at risk.

The need for Pathophysiological Studies.

It is clear that death in cholera is due to loss of water and electrolytes in the stools. It can be prevented if fluid and electrolyte balance is restored and maintained for three to five days, during which time spontaneous recovery ensues.

There are several transport systems for electrolytes in the intestine and it is clear that one of them is affected by cholera toxin while at least two others are unaffected. These last are the glucose-associated and glycine-associated pathways for Na absorption and both of them have been exploited in the oral regimes developed and reported.

Further advances in therapy are most likely to arise from the following:

A. 1. The identification of the toxin-affected pathways at the cellular and at the subcellular levels.

2. The dissection of the chemical processes involved in its action.

3. The uncoupling of toxin or bypassing of the enzyme step or steps affected by it.

B. The investigation of other pathways, for electrolyte absorption unaffected by toxin, to determine whether they can be exploited in the same way as has been done with the glucose and glycine-associated pathways.

The Role of the PSCRL.

We have been asked to list priorities for the work of the Laboratory but we believe that it is inappropriate to do so. Any such list or direction of the research would restrict the freedom of action of the Director and staff who, we believe, should be free to decide on their own priorities.

It is self-evident that the principal effort should be centered on the cholera patient and that the seasonal nature of cholera requires that the months between outbreaks should be used for laboratory and animal experimentation. Beyond endorsing these points already made in Dr. Hare's Report of the Physiology Division, we would only comment on the continuing high standard of the work presented and in progress.

We are pleased to see the progress that has been made in particular in two directions as follows:

A. As a result of the completed flux studies and an ongoing study of the colon in cholera (Protocol 04 70 04) the macroscopic localization of the lesion in cholera is becoming clearer.

B. The oral regime, as proposed earlier and subsequently modified by the addition of glycine, has been demonstrated to be effective (Protocol 04 70 07). We hope that this work will be extended to the study of other pathways of Na absorption than the glucose and glycine ones.

In the case of the current studies we would single out two for comment:

1. Protocol 04 70 01 - Studies of material obtained by Crosby Capsules from patients with cholera and using the Ussing technique.

Dr. Rohde has already shown that the method is capable of obtaining useful and unexpected information. We hope that he will extend the work to embrace isotopic fluxes and the study of animal material after known intervals from exposure to cholera toxin or live vibrios. Further expansion of the work in other directions would be commended.

2. Protocol 04 70 02 - Distribution of body water and fat.

The preliminary data presented to us on cholera patients show that certain individuals have grossly abnormal distributions of body water and fat. These are almost certainly unrelated to cholera itself but are a manifestation of malnutrition or other disease. Although the most interesting results lead away from the main stream of cholera research we believe that the subject should be pursued at least for the time being.

EPIDEMIOLOGY DIVISION

Vaccine Field Evaluations.

The severe epidemic of cholera in the Matlab study area during the 1968-69 season made possible the most definitive vaccine evaluation in the history of the PSCRL. Monovalent whole cell vaccine, Inaba type, conferred marked protection of better than 90% for a period of at least three months. A purified Inaba antigen was equally effective. A monovalent whole cell Ogawa vaccine failed to protect. This field trial revealed, for the first time, that immunity against classical Inaba cholera is type-specific.

The participants in the field trial have received booster inoculations and will be observed for a second year in accordance with the original plan for the trial when it was established.

Cholera due to El Tor Ogawa strains is presently occurring in the study area, thus giving promise that a test of both type-specific immunity from Ogawa vaccine and heterologous immunity from Inaba vaccine may be obtained this season.

Substantial progress has been made during the past year in the production and purification of cholera toxin. There is a good prospect that an effective toxoid will be available for large-scale field trials during the 1971-72 seasons. In the meantime, it is recommended that the present study populations be kept under observation to measure duration of immunity of the vaccines presently under test. Plans should be formulated for a large-scale field trial with pure toxoid and a combination of toxoid and bacterial antigens as soon as these products become available. The old test area will be most suitable for inclusion in this field trial because a population of approximately 25,000 unimmunized children under six years of age will have accumulated by 1971.

Serological Studies.

The purification of cholera toxin has led to the discovery of a new in vitro hemagglutination test for antitoxic antibodies (Finkelstein and Hochstein). The preliminary experiments at PSCRL with this test reveal it to be reproducible, sensitive and specific. It is recommended that this test be further developed and fully evaluated in a variety of epidemiological studies with the view of having it available as a routine method by the time the toxoid field trial is initiated.

Field Epidemiology.

The intensive epidemiological studies in the village of Meharan in the Matlab area during the 1968-69 season revealed the extent of inapparent infection during an epidemic of classical Inaba cholera. Among 1,600 members of the village, 116 persons became infected, but of them only 11% required hospitalization and 15% treatment as outpatients. The majority of infections were asymptomatic. It is recommended that these studies be continued, especially in view of the recent recognition of the presence of El Tor Ogawa infections in this area.

The studies of cholera in Chittagong during the 1968-69 season demonstrated the value of maintaining at least limited observation of cholera in areas of East Pakistan other than Dacca and Matlab. A comparison of the clinical and epidemiological characteristics of classical Inaba and El Tor Ogawa infections was accomplished. While clinical cases of the two types of infection were indistinguishable, there were distinct epidemiological differences. The carrier rate among family contacts was higher for the El Tor biotype than for the classical strain, and the El Tor strain was isolated ten times more frequently in nightsoil and latrine samplings. It is recommended that the surveillance of cholera infections in Chittagong be continued.

Demographic Studies.

1. Descriptive Demography. Analyses of rates of births, deaths, stillbirths, neonatal mortality, and in and out migration among the 117,000 persons in the "old test area" at Matlab have been completed for two consecutive 12-month periods and preliminary data for a third year are available. Additional data for one year among the 110,000 persons in the new test area are also fully recorded. Thus, a population of 227,000 persons is now available for continuing descriptive demographic studies.

These studies, which up to the present, have been a mere by-product of the vaccine evaluation program, have attracted the attention of the Pakistan government, the AID, the National Institute of Child Health and Human Development (NICH&HD) of the NIH in Washington, the Population Council and the Ford Foundation. These demographic data may well represent the most precise and detailed figures available anywhere in the subcontinent.

The NICH&HD-NIH has agreed to assign a demographer to the PSCRL to assume responsibility for extending and developing this demographic resource. A collaborative project with AID-Pakistan and Dacca University has been prepared and is now being processed. It is recommended that this project be given full support and be incorporated as a continuing activity of PSCRL.

2. Maternal Mortality. In the latter half of 1968 a study of maternal mortality in the Matlab area was undertaken in collaboration with the East Pakistan Research and Evaluation Center. A maternal mortality rate of seven per 1,000 live births was measured. This is considerably lower than recorded rates from other areas of the subcontinent which are derived from hospitalized patients. This study is now complete and has been reported at several conferences. It illustrates the value of the Matlab population for broad demographic and epidemiologic studies.

3. Pilot Pregnancy Wastage and Interval Study. In January 1969 a new collaborative project, supported by AID-Pakistan and the Population Council, was undertaken in Matlab to determine the feasibility of using pregnancy tests and detailed interviews of women in the childbearing age groups to measure pregnancy, abortions, fetal wastage and child spacing. A microtest on urine was developed in the PSCRL reducing the cost per test from $1.00 to $.20.

The cooperation on the part of the women in this population was excellent. They could be readily classified into three main groups on a monthly basis:

a) menstruating women 50 to 60 percent

b) lactating women 30 to 40 percent, and

c) pregnant women 10 to 20 percent

Only a small fraction of women were found to be sterile.

Over the first six month period of study new pregnancies occurred from four to five times more frequently among menstruating women than among lactating women, confirming a long-held belief which has only rarely been documented.

This pilot project has established the feasibility of conducting a large-scale investigation of pregnancy, pregnancy wastage and child spacing in the Matlab study area. AID has continued support of the pilot project for six months and is now considering a formal project proposal. It is recommended that PSCRL collaborate in this proposal with the understanding that:

1) adequate funds will be made available

2) appropriate statistical and computer programing services can be provided

3) regular consultation with a qualified research gynecologist can be obtained, and

4) the project has the full endorsement of the Pakistan government

5. Other Epidemiological Studies.

During the six years of its existence the Matlab population has been considered for a number of epidemiological studies other than cholera but on only rare occasions have these been undertaken because they were not considered to be appropriate to the mission of the Laboratory. In 1967-68, during a period when cholera was absent in Matlab, a collaborative study of smallpox was undertaken with the WHO and the Pakistan Medical Research Center in Lahore. Age-specific vaccination effectiveness rates were measured with great precision. These ranged from 94 to 96% in children to 74% in adults.

It is recommended that other epidemiological projects be judiciously considered for appropriateness to the Matlab situation. For example, diphtheria-tetanus toxoid has been employed as a placebo inoculation in the cholera vaccine studies. Tetanus neonatorum is known to be a serious continuing health problem in East Pakistan. It would be a simple procedure to evaluate tetanus toxoid in the prevention of tetanus neonatorum as an addition to the proposed cholera toxoid field trial in 1971-72.

Other diseases should also be considered. Measles is generally recognized in the community, but a pilot project would need to be undertaken to calibrate the degree of accuracy of the present lay staff in collecting such reports. Nutritional conditions, such as edema, or kwashiorkor may also be feasible for study. More sophisticated nutritional studies would require the addition of trained personnel.

Signed by the members
of the Technical Committee

(Dr.Colin M.MacLeod, Chairman)

(Dr.Ziaur Rahman)

(Sir James W. Howie)

on December 4, 1969 Dacca, East Pakistan.