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REPORT OF THE ELEVENTH MEETING OF THE SCIENTIFIC
REVIEW AND TECHNICAL ADVISORY COMMITTEE
OF THE CHOLERA RESEARCH LABORATORY
DACCA, BANGLADESH

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Report of the Eleventh Meeting of the Scientific
Review and Technical Advisory Committee
of the Cholera Research Laboratory
Dacca, Bangladesh
February 22-March 1, 1977

INTRODUCTION

The Scientific Review and Technical Advisory Committee (the Committee) of the Cholera Research Laboratory (CRL) met for one week in Dacca, Bangladesh, to review the present and proposed activities of the CRL, and to provide technical guidance to the Directing Council. The members of the Committee, Consultants, and Observers are listed in Attachment No. 1. The Agenda for the meeting is summarized in Attachment No. 2.

The Committee noted with regret that it was meeting in the absence of the Director, Dr. Willard F. Verwey, who had returned to the United States two months earlier because of illness. The Committee extends its best wishes to Dr. Verwey for a speedy recovery, and commends him for his leadership of the CRL since its re-establishment as a research organization in May of 1974. In his Annual Report for Fiscal Year 1976, Dr. Verwey observed that: "It is a source of satisfaction, but not complacency, to state that over this period of time, particularly in FY 76, the Laboratory has moved from a situation of gross underutilization to one where it is now a matter of concern that the research projects are competing with each other for both personnel and facilities." The Committee shares both this satisfaction and concern.

The Director's Report comments on the re-orientations of CRL research activity that have occurred in response to developments in diarrheal disease research programs in other laboratories and in recognition of the needs of Bangladesh and other developing countries. Dr. Verwey's listing of these trends serves as an appropriate introduction to the Committee's appraisal of the research program:

1. Greater emphasis on work with the non-cholera diarrheal diseases.
2. A corresponding reduction in the preoccupation with cholera per se.
3. An intensification of the interests of the Laboratory in problems of population control and fertility.
4. An increase of interest in the inter-relationships between nutrition, diarrheal disease and fertility at the biomedical level.
5. In assigning research priorities, a somewhat greater weight applied to research offering possibilities of interventive action."

The Committee also noted that its meeting not only coincided with a change in CRL Directorship, but was being held at a time when negotiations, authorized by the Directing Council in August, 1976, were in progress to convert the CRL to an independent international institution having multilateral financial and technical support. Because this development bears directly on the future research program of the CRL and its successor Institute, a brief summary of the status of the conversion, now targeted for October 1978, has been included in this report.

The work of the Committee was greatly facilitated by the participation of Dr. Henry Mosley, Director Designate, of Dr. William B. Greenough, III, who is to become Director of Clinical Science, and of Dr. Michael Merson, who has been deputed by the Center for Disease Control to succeed Dr. George Curlin as Head of the Epidemiology Division. These three investigators have had prior experience at the CRL, and a smooth and effective transition in leadership seems assured.

The Committee expresses its gratitude to Dr. M. Mujibur Rahaman, Acting Director, for his gracious hospitality, and thanks the entire staff of the CRL, particularly Dr. George Curlin and Mr. Philip Weeks, for facilitating the review process. Both the Committee and the staff benefited from the participation of the consultants, all of whom contributed to the preparation of this report.

The scientific activities of the CRL are reviewed in the following sections in the order in which the research programs were presented by the responsible investigators (Attachment No. 2). In addition, to assure continuity with last year's Committee review and to focus on administrative concerns as they may bear on research productivity, brief sections are devoted to all Divisions other than Maintenance.

RESEARCH PROGRAMS

Oral Replacement Studies in Diarrheal Diseases

Diarrhea constitutes one of the most common and debilitating health problems in Bangladesh. Data from the CRL indicated that in 12 villages during November - December 1975, there were 67 cases of diarrhea per 1,000 person months. Although rates varied, the average incidence of diarrhea was one episode per person per year. About one per cent of cases required hospitalization. Both the incidence and case fatality due to diarrhea were much higher in young children than in adults. Replacement of fluid and electrolyte losses with a solution that may be taken orally has been shown to be effective in treating cholera and related diarrhea under medical supervision. This mode of treatment is very simple and inexpensive and could be applicable on a wide scale.

As proposed to the Committee in 1976, a trial of initial oral rehydration was done in the out-patient service of CRL, with continuing replacement being completed at home. From the approximately 200 patients who attended the OPD each day, cases deemed by objective criteria to be moderately severely depleted as defined by a plasma specific gravity of

1.029 were entered in the study. Of 451 consecutive cases, 174 had specific gravities less than 1.029, and were excluded. Of the 277 remaining, 230 were successfully rehydrated in the clinic and at home. The remaining patients required hospitalization or supplementary intravenous fluids. Of those patients followed at home, six required additional intravenous treatment. Of the 174 milder cases, 12 were considered treatment failures. These data indicate that oral replacement with a special solution in an ambulatory care setting followed by unsupervised treatment at home may be a highly successful and inexpensive approach to treatment; since there was no concurrent control group treated with kaolin mixture, this conclusion required confirmation.

In four villages of the Matlab area a subsequent study was conducted on the usefulness of unsupervised oral replacement therapy for diarrhea. In two villages cases were treated with the standard aqueous kaolin mixture; in two other villages, with the CPL oral replacement solution with the following composition: Na^+ , 120; K^+ , 25; Cl^- , 9; and HCO_3^- , 50 mEq/l, and 20 grams per liter of glucose. Dried salts were packaged to be mixed in a seer* of water. Samples of the mixtures used were assayed later to verify how accurately families prepared the solution. All patients were advised to come to the Matlab Hospital should they feel treatment was not working. Field Assistants visited all cases daily, and took blood specific gravity measurements on the first and third days. Serum sodium was also measured on the third day. All cases were cultured for etiologic diagnosis.

Except for the occurrence of 10 cases of cholera in the standard treatment villages as compared to only one case in the special treatment villages, the pattern of diarrhea was comparable, with an incidence of 20 cases per 1,000 person months. Too few severe cases were seen to judge effectiveness in the prevention of serious morbidity. In the mild cases both forms of treatment worked equally well, but in the oral replacement group there was a significantly greater reduction in blood specific gravity on the third day. There was great variability in composition of the home mixed solution; sodium concentrations ranged from 90 - 230 mEq/Liter. When the Field Assistants showed families how to measure one seer, the accuracy of mixtures was much improved. There was one instance of hypernatremia, presumably due to inadequate intake of salt-free water.

During discussions of these results, it was suggested that to ensure safe and effective use of oral replacement solution in the home, several important steps must be taken. First, it will be necessary to establish a convenient and accurate measure of the amount of water to be added to packaged dry salts by an uneducated person. Second, the salts must be packaged cheaply, yet secure from moisture. Third, the acceptability of the salt solution must be established with respect to color and flavor. Fourth, it must be determined whether prior training or supervision by a health worker is needed. Fifth, it must be shown that

* Seer—a poorly standardized measure, approximately a liter, but varying with the size of available container.

mortality and the need for hospitalization can be averted in a field situation. Finally, it will be necessary to demonstrate the superiority of oral electrolyte therapy over water and food ad lib term. To do this, means must be devised of assuring that the home-mixed solutions are properly prepared.

E. Coli Diarrhea-

An excellent study of the clinical and laboratory aspects of diarrhea associated with toxinogenic E.coli (TEC) was carried out on 176 males over 10 years of age admitted to CRL with acute diarrhea during 10 weeks in the fall of 1976. Cholera and dysentery-like illnesses were excluded. Previous data had shown that the usual enteric pathogens could be isolated from less than 50% of patients admitted to the Matlab hospital. The purpose of this study was to determine how many of the remaining "unknown" diarrheas were associated with TEC and to look for clinical and epidemiologic correlates with these agents. Heat labile toxin (LT) was detected by the Chinese hamster ovary cell assay, and stable toxin (ST) by the suckling mouse assay. Both assays appeared to be well established at the CRL.

Sixty-two percent of the cases yielded either LT or ST producing strains on selective culture. More than half were LT-ST; one-third were ST only; six strains were LT only. Only one isolation of invasive E.coli was made. A few non-cholera vibrios were isolated but usually in conjunction with TEC.

E.coli diarrhea was relatively much more common among adults than is cholera. Clinical analysis suggested that LT-ST disease was slightly more severe than diarrhea associated with ST-only. ST-only cases had significantly shorter duration of stooling and lower stool volume; this is consistent with the activity of ST in animal models. There were too few cases of LT-only to draw conclusions. A controlled study indicated that tetracycline was moderately effective in reducing the duration of LT-ST disease, but had no effect in ST disease. This important observation should be followed by a search for association between ST and RTF plasmids.

Excellent data on the effect of pooling clones for LT detection showed that, in clinically manifest disease at least, picking of one clone was as sensitive as testing a pool of five or 10 clones.

The investigators plan to measure anti-LT antitoxin response, especially in relation to antibiotic therapy, to look for persistent infection following clinical recovery, and to attempt direct LT and ST detection in stool samples. Further studies on antibiotic sensitivity in relation to toxin production are also planned. Rotavirus studies will be carried out on stored serum and stool samples.

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It will be important to learn how much inapparent infection there is with enterotoxinogenic E.coli. The effect of pooling of isolates will be an important consideration. Because of the immunologic relationship between cholera and E.coli LT toxins, sero-epidemiologic surveys of antitoxin levels to either toxin may be useful in clarifying the epidemiology of both cholera and TEC diarrhea in Bangladesh, and might provide an understanding of how they relate to one another.

Epidemiologic data collected in Matlab during 1974 and 1975 sought to determine the role of LT+ E.coli in the total diarrheal disease picture. Diarrheas of unknown etiology accounted for 58% of 11,982 cases studied. The case fatality rate in this group was 1.1% in contrast to 5.6% for cases of shigellosis. Disease appeared in spring and fall peaks roughly comparable to cholera. E.coli strains isolated during the latter part of the study were tested for LT production, and about 18% were found to be positive. The isolation rate of LT+ strains was more than twice as high in hospitalized cases as in OPD cases. It was estimated that the overall incidence of LT+ diarrhea which reached Matlab facilities was about 1.7/1000 during a six month period. The rates were highest in children aged 1-4 years, but their illnesses tended to be mild and seldom required hospitalization. The disease became more severe with advancing age; 70% of persons over 15 required hospitalization. This well-designed and well-executed study has provided the first quantitative estimate of the impact of diarrhea associated with TEC in this population. It is hoped that similar data can be obtained on ST disease, and on inapparent infection with organisms producing both LT and ST.

Amoebic Dysentery

A study has described the appearance of the rectum during clinical amoebic dysentery as viewed through a proctoscope. Cases were selected on the basis of blood and mucus in the feces associated with E. histolytica on microscopy of fecal smears. A characteristic pattern emerged in that the earliest visible signs were excessive mucus and swelling of the rectal folds, followed by a lobular appearance in the pink mucosa due presumably to changes in the vascular bed. With suitable treatment these changes could be reversed. A serious complication was the beginning of necrosis in the bowel wall; this signaled a poor prognosis even with optimal treatment. Even in those patients who did recover, the appearance of the rectum returned to normal only after a long delay. The necrotic form appeared to be a different syndrome in that it had a much more acute onset and occurred exclusively in older people. On the basis of this study, cases are now clarified in one of three categories depending on degree of severity of the proctoscopic findings: - mild, lobular, and necrotic. An interesting feature was that severity did not seem to be related to the numbers of E. histolytica found in the rectal swabs.

Two other positive observations were made. When delayed hypersensitivity to a variety of antigens was tested, the mild and lobular forms exhibited similar proportions of positive skin reactions. By contrast, those patients with the necrotic form showed a remarkable and significant depression in their D.H. reactions. Another finding which correlated with necrosis was the level of a serum protein component called prealbumin. On admission to hospital the prealbumin was lowered to about 10% of normal in those patients with necrotic lesions. It is not clear at the moment what the functional significances of these two correlations are, but they are both of considerable interest and further work is required. The Committee thought this work was good, but suggested the inclusion of a control series to determine the prevalence of E. histolytica infection in the general population. Since there was no correlation between severity and numbers of E. histolytica, discussion centered on the casual role of this organism in the production of the rectal changes. For this question it would be useful to know if increases in antibody levels to E. histolytica antigens occur during the course of the disease.

Leukemoid Reaction in Shigellosis

Dysentery due to shigella infection continued to be a major clinical problem at CRL in 1976. More than 1,100 cases were admitted. These had a six per cent case fatality rate, with the rate being highest in young children as usual. The interesting leukemoid syndrome which was described at CRL a few years ago was again a feature in 77 cases. As a result of intensive investigation of such cases, it seems that endotoxin may circulate in the blood stream of patients present with the hemolytic-uremic syndrome, and that those with a leukemoid reaction may possess circulating immune complexes. As a working hypothesis, it has now been suggested that these complications follow the absorption of bacterial endotoxin as a result of severe colonic ulceration. This bacterial antigen then evokes a brisk immune response; at a certain stage in antibody formation, immune complexes may form, circulate and be deposited, particularly in the kidney. This simple hypothesis is at least testable. Preliminary studies indicate that when immune complexes are detectable endotoxin is not. It is therefore likely that the immune complexes are in antibody excess. Thus, it should be possible to detect anti-shigella antibodies at this time, - perhaps by using the hemagglutination test. No doubt this will be done. Real progress has been made in the last year towards resolving the puzzle of this interesting condition.

Shigella toxin

Work on shigella toxins has not advanced much since last year's report was written. The existence of Shiga toxin in filtrates of local strains has not yet been clearly demonstrated. A few studies have been carried out on rabbit ileal loop activity in crude filtrates from one selected strain of Shigella dysenteriae type 1. As expected, shigella filtrates are relatively less active than cholera toxin. Membrane filtration suggests a molecular weight of 50,000 - 100,000 which would be

consistent with shigella neuroenterotoxin. Attempts have been made to raise antitoxin in rabbits, but this has shown only minimal protection in high doses. The toxin appears to be stable at 56C but destroyed at 99C.

Although it was recommended last year that toxin produced by the local strain be tested against the International Standard Dysentery Antitoxin this has not been done. It is important to determine whether the exotoxin(s) elaborated by local strains are immunologically related to classical Shiga neurotoxin and to the toxin produced by recently isolated strains from Central America. HeLa cell studies have been dropped, and no attempt has been made to look for antitoxin response in dysentery cases.

Since the pathogenesis of the leukemoid-hemolytic syndrome in shigellosis is still unknown, it would be wise to determine the antitoxin response in these cases, and to look for evidence of involvement of the Shiga exotoxin or derived antigens in the pathophysiology of this disorder. A mouse assay has been described and it was recommended last year that this be used at CRL. Examination of the effect of toxin-containing preparations on blood elements of laboratory animals might also be informative.

Procedures for simple concentration and partial purification of Shiga exotoxin were described long ago, and could be used to advantage at CRL to help answer a few of these basic questions without getting involved in sophisticated purification techniques. If preliminary work with Shiga toxin should suggest promising ways of relating toxin to the pathogenesis of shigellosis, then these studies might be pursued further; to be successful, however, more direction and greater collaboration among members of the CRL staff will be necessary.

Diarrhea Associated with Non-Cholera Vibrios

There has been a recent and sudden increase in the number of diarrhea cases associated with the isolation of non-cholera vibrios of Heiberg Group III. Very preliminary data were presented showing that crude culture filtrates of these organisms caused fluid accumulation in rabbit ileal loops. The filtrates have not been tested for heat stability or resistance to trypsin. Since previously described NCV toxins have been cholera-like, it would be sensible to determine the effect on vascular permeability in skin and on CHO or adrenal cells, and to do neutralization tests with cholera antitoxin. Also, the strains isolated should be sent to a reference laboratory for serotyping so that meaningful epidemiologic data can eventually be obtained. The data presented mean very little as they stand, but a systematic study of non-cholera vibrios, which appear to be agents of diarrhea would be worthwhile. Such studies would include:

determination of PF activity in filtrates of non-cholera vibrios isolated from different sources to look for association between toxinogenicity and pathogenicity; confirmation of positive PF tests by ileal loop and neutralization by cholera antitoxin; preparation of antitoxin against immunologically unrelated "new" toxins if they are found; serotyping of representative strains; measurement of antitoxic and specific antibacterial serologic responses in patients from whom organisms were isolated.

Population Studies

The data which have been carefully recorded for the vaccine trial area population of 260,000 persons since 1966 provides demographic information of increasing value each year. Serious disruption of the population over a nine month period occurred as a result of civil unrest in 1971 and again in 1974 when a serious food shortage followed floods and failure of the rice crop. Mr. Alauddin Chowdhury reported the effects of these events on certain demographic markers. Death rates over the period 1966 to 1971 were relatively stable; in 1971 there was a 33% increase over previous levels. In 1974, there was again a sharp rise, about 50% over the previous year. Infant death rates showed a similar picture, with a more distinct upward trend. Increased numbers of deaths were mainly among the children and the elderly; the increased mortality was associated with a markedly increased incidence of shigellosis. The total fertility rates showed a very slow downward trend attributable to a pronounced decline in the age-specific fertility rate of the 10-19 year age-group. A fall in 1975 affected all groups. The validity of these data was confirmed by comparing "Old Trial Area" (those villages studied since 1966) and the "New Trial Area," (those villages added in 1968); the curves were essentially identical.

To test the hypothesis that the declining fertility in the 10-19 year age-group was due to later menarche and later marriage, girls in this age-group living in 13 villages were interviewed. There had been an increasing age of onset of menarche, and this appeared to be associated with the malnutrition experienced during the period 1971 through 1975. Higher body weight was strongly associated with earlier age of onset of menarche, which was in turn associated with earlier age of marriage. Data for Hindu girls was comparable to those for Muslim girls, although their age at marriage was somewhat higher.

To study the relation of maternal nutrition to the length of post partum amenorrhea and to the interval between resumption of menstruation and conception, a longitudinal study of 2,200 married women aged 15-49 years was begun in November 1975. Analysis of the experience of the first year indicated that the duration of post partum amenorrhea was longer for women with lower body weight (26 vs. 22 months); the interval between resumption of menses and conception was even more strikingly

lengthened (40 vs 24 mo.) in these women. Another survey of over 2,000 women found that the median length of post partum amenorrhea in breast-feeding women was 20 months, and that the period of amenorrhea was prolonged with increasing age and/or parity. Higher socio-economic levels were associated with shorter intervals of amenorrhea. Improved nutrition of the mother, measured as ponderal index, decreased the period of amenorrhea; supplementation of the infant's diet reduced the proportion of post partum women who were amenorrheic. These findings indicated a direct relation between nutrition and fertility. This was supported by the interesting practical observation that there was a marked seasonality in the resumption of menses, with a greater proportion of women resuming menstruation in November and December following the harvest, regardless of whether the birth occurred in February-April, or August-September.

A survey of 3,850 married women (in 20% of the villages in the area now included in studies on contraceptive dissemination) compared the number who became pregnant during the famine with the number who became pregnant one year later. After the famine, increased fertility was manifested by the poorest women. Poverty was estimated by possession of minimal living space, and by marriage to landless agricultural laborers. Thus, fertility was markedly reduced in those most affected by the famine.

A contraceptive distribution project was described in which contraceptives were made available at the doorstep in over 100 villages with a population of approximately 130,000; a comparable area served as a control. In this project the cholera program Lady Village Workers (dais) and Field Assistants brought the materials and instructions to each household. A preliminary survey indicated that 2.4% of women were using a modern contraceptive method, and 4.9% traditional methods. Thirty-six per cent indicated a willingness to use contraception, and 25% of these would use pills. The second choice was periodic injections; none chose IUD's. For a 20% sample of women, experience in the first year showed that contraceptive use rose from 1.1% to 18%, falling at the end of the period to 15%. In contrast, use in the control area was reported by 2.9% at the beginning and 3.6% at the end. The higher basic rate in the control area is explained by inclusion of Matlab village where the Johns Hopkins Fertility Research Project is functioning. Use was greater by Hindu women than by Muslim women in the control area, and comparable in the study area. The effectiveness of the program in terms of lowered birth rate was evidenced by a lowered proportion of women who reported pregnancy in the contraceptive area than in the control area in the surveys carried out after April 1976. By a case-control method, symptoms in users were compared to those in non-users from the same village and from a control village. Dizziness, eye problems, and a "burning sensation" were reported more frequently by users. Breast feeding was practiced by significantly more contraceptive users; non-users more frequently wanted more children. Of interest was the fact that 61% of users and 34% of non-users would be willing to undergo induced abortion at two to three months gestation.

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These studies have provided basic information of importance to any family planning operation, and have been made possible by the quality of demographic information assembled by the Cholera Research Laboratory, and by the presence in these communities of CRL health workers. Such data should also be of use to the Bangladesh government.

Environmental Epidemiology

Environmental studies have expanded into new directions with the objective of determining the role of ecological factors, behavioral characteristics, and sociological determinants in the transmission of enteric pathogens and their persistence in the environment. The Committee heard six presentations, five dealing with studies completed or in progress, and one dealing with plans for a study in progress. A study of the relationship between water consumption and dysentery in Teknaf, a rural Bangladesh village, was designed to assess the relationship between the quantity of water used and the risk of developing clinical dysentery and/or laboratory confirmed shigellosis. The quantity of water carried to each family from a communal well was estimated in two communities in 1976 during a period of surveillance for diarrhea. There was an inverse relationship between the availability of water and the attack rates for clinical dysentery and shigellosis. An impressive reduction in both rates occurred when the amount of water increased from less than 20 to 30 or more liters per person per day, this in spite of the fact that the water was presumed to be contaminated. Presumably this improvement was due to the fact that larger quantities of water facilitated washing and cleaning of body surfaces and eating utensils - thus the term "water-washed" in referring to shigellosis and dysentery. This important study has demonstrated quantitatively, perhaps for the first time in the Asian subcontinent, the substantial impact of water availability on a water-washed disease.

Information concerning the comparability of the three study groups in this investigation was not available at the time of presentation. It will be important to validate these findings by demonstrating that the groups are comparable with regard to age stratification and socio-economic status. The investigators were also encouraged to pursue the opportunity available to them to compare attack rates not only among those who have water carried to their homes but also among those who have water near or inside their homes. If this can be done quantitatively, it may further demonstrate the value of water sources close to and in homes, and may also provide information on the optimum quantity of water necessary to give a maximum effort for control of a water-washed disease. Finally, the data showed considerable difference between the clinical dysentery attack rate (255/1,000/year) and the laboratory confirmed shigella attack rate (43/1,000/year) raising the possibility that etiological agent(s) other than shigellae might be operative.

A questionnaire study was designed to assess health consciousness, hygiene practices and attitudes in Teknaf to provide information that presumably would serve as a base-line and guide in health education and

in directing future sanitary improvements. It is uncertain how this information will be used. It would seem logical that the survey be repeated to assess the impact of an intervention such as intensive health education.

A two-year study of the efficiency of hand-pumped tubewells in the prevention of cholera and shigellosis has been conducted in rural Matlab. It was established that tubewell water was used for drinking purposes only, and that there were other sources of water for washing, cooking, etc. Users of tubewell water were not protected, for their attack rates were comparable and in some months significantly higher than rates among persons who used other sources of water. While this is a disappointment to some who had hoped for opposite findings, it is not surprising considering the fact that tubewell water does not serve as a substitute source but rather as an additive source of water. The message seems clear: transmission of these diseases will continue as long as people are exposed to contaminated water sources. The challenge is to find ways to motivate people to avoid these sources or to clean the sources.

A study of water and foods consumed by cholera patients and their family members was conducted to determine how transmission occurs in cholera. Family members of cases admitted to Matlab hospital, items of food, water for cooking and other uses, and environmental surfaces were cultured for ten days. Qualitative and quantitative assays were used to clarify the chain of transmission, and to determine whether there is a multiplication step between the contaminated source to the vehicle of transmission. A multiplication step could not be demonstrated. The study further showed that food did not appear to be responsible for transmission as only two of 1,511 food specimens were found contaminated with low numbers of V. cholerae. Evidence pointed to water as the major vehicle of transmission. Perhaps the most important finding was the low level of contamination in water, suggesting that low-inoculum exposure in cholera may be the rule rather than the exception. These water contamination findings are considered preliminary, as only 13 sites have been studied. The study should be continued along the same lines until a total of about 100 sites are available for analysis. If the completed study shows findings consistent with these preliminary data, this would heighten interest in those host factors which predispose to clinical disease.

A basic environmental study has been commenced at Meheran to characterize two types of water systems and to determine the biological cycles of micro-organisms, including V. cholerae. The water systems are a tank, which is a static system with marked layering, and a canal, a non-static system with no layering. An interesting preliminary finding was the apparent concentration of V. cholerae, probably by adherence, by the water hyacinth. The interaction of V. cholerae with water hyacinths and other plants will be studied in the laboratory. This is potentially a very valuable study, not only in revealing new knowledge of the life cycle of V. cholerae in the external environment, but also in providing new information of two types of water systems important to the citizens of Bangladesh. Drug resistance and plasmid analysis of human enteric organisms (obtained in this study) is also contemplated.

Plans were presented for a study of water use and defecation behavior. The study, now in its initial phase, has two complementary components. The first component consists of studies on traditional water use and defecation behavior and an analysis of factors which may influence any proposed interventions. The second component is the intervention step that will emerge from this analysis. The Committee liked these plans.

ADMINISTRATION: BACKGROUND AND TRANSITION

The Cholera Research Laboratory (CRL) was re-established in Dacca, Bangladesh with the signing of a bilateral Project Agreement between the Governments of Bangladesh and the United States on 15 May 1974. The Agreement provided for expansion into areas of biomedical research other than cholera and diarrhea, such as demographic studies and interaction between the malnutrition and infection. Although the project was to be funded by the two governments, provision was made to encourage other governments or organizations to contribute support. The Governments of Australia and Great Britain have responded to this opportunity by providing cash or materials and equipment needed by the Laboratory each year. These two countries had also contributed to the support of the CRL before the change in government in 1971.

The CRL remained intact during this change, although it functioned primarily as a service activity for Bangladesh, treating patients with diarrhea at its hospitals in Dacca and at the field station in Matlab Bazaar. Dr. George Curlin, an Epidemiologist from the Center for Disease Control in the U.S., served as Interim Director, and prepared the staff and field study area for resumption of research activities. Dr. Willard F. Verwey from the University of Texas Medical Branch at Galveston came to Dacca as the first Director of the reconstituted CRL. He served in this capacity from 1 June 1974, until he retired on 28 February 1977.

Dr. Verwey reorganized the CRL after his first year as Director into five new Divisions: Administrative, Clinical, Epidemiology, Laboratory and Maintenance. The staff currently consists of 535 Bangladeshi and seven expatriates. The expatriates are five scientists including the Director, a special assistant to the Director and a maintenance officer. Two of the scientists are epidemiologists provided by the Center for Disease Control, while the remaining expatriate employees are hired by contract. A biochemist from Britain will join the staff in the spring of 1977. The Johns Hopkins International Center for Medical Research is located in Dacca; its staff of two to four scientists conducts research of mutual interest, reimbursing CRL on a fee-for-service basis.

Dr. Wiley H. Mosley, formerly Chairman of the Department of Population Dynamics at Johns Hopkins University and formerly Chief of Epidemiology at CRL in the 1960's, became the new Director on 1 March 1977.

The present Project Agreement is due to expire on 30 September 1977. Negotiations are underway to extend the bilateral Agreement to September 1978, to allow time for converting the CRL into an independent

international institution with a broad base of support for more activity in several areas of medical research important to developing countries. The U.S. Agency for International Development (USAID) recognizes the unique resource of the CRL field population of 260,000 persons with census data dating back to 1965. No population of comparable size exists in a developing country where it is possible to do rate based studies. With the assistance of Drs. Verwey, Mosley and Lincoln Chen of the Ford Foundation, a five-year plan was developed and a prospectus drafted for developing such a new international institution. The concept has been accepted in principle by the Inter-ministerial Committee of the Government of Bangladesh and by USAID. The Ford Foundation has provided a grant to aid in the development of a charter under the laws of Bangladesh. The Governments of Australia and Great Britain are considering continuation of support. A final prospectus is being drafted to use in seeking other donor support. The USAID will continue as a major donor for several more years, but expects to reduce its percentage support as funds are available from other donors. The name of the CRL will be changed to reflect its expanded range of bio-medical research; International Institute for Health, Nutrition and Population Research has been suggested. Plans for the transition seem to be progressing well.

CRL DIVISIONS

Administrative Division

The Administrative Division has made a number of improvements in the past year. Most of these center on the computerization of some records and development of a cost accounting system. This accounting system allows the charging of a fee-for-service to CRL protocols as well as to cooperating and non-staff protocols. There are still some defects in the system, and some protocols still do not have a cost figure.

The present staff is handling all activities well. Mr. Philip Weeks has been particularly helpful as Administrative Assistant to the Director, and has worked effectively with the administrative staff. His work deals mainly with AID and other activities outside the CRL, while the administrative staff is concerned with activities within CRL.

The Supply Branch has moved to renovated quarters in the storeroom. Most of the bulk storage was moved to a warehouse to make room for the staff. All items in supply have been indexed according to the NIH system, and the inventory placed in a computer. This should be a great improvement over the old card system. The usual delays in getting supplies from the U.S. still occur. Fortunately, there have been fewer problems in the last few months. Also, the ability to purchase in Singapore and other places has been a great help.

The CRL Library is making a real effort to be responsive to the needs of scientists in and around Dacca. It maintains close liaison with the National Library of Medicine, housed in another wing of the Institute of Public Health. The limited resources of each are coordinated to avoid unnecessary duplication of books and journals. The CRL has access to Medline literature searches through the National Library of Medicine in the United States. This cooperation is to be commended.

Clinical Division

Seven items of investigation were projected in the 1976 report of the Committee, and now call for some comment.

Studies on all aspects of shigellosis have been pursued. In particular, further studies of the leukemoid reaction and hemolysis have led to the formulation of a hypothesis regarding the pathogenesis of these complications, which will be tested by continued studies. The shigellosis research unit is functioning in as far as there is a separate 20-bed unit, with its own nursing and clinical medical staff, and record system. Studies on alternative drugs for ampicillin-resistant shigella have been concluded. Trimethoprim-Sulphamethoxazole has been found effective.

A comparative trial of Dacca and WHO solutions has been completed and results published. There appears to be little difference in efficacy between the solutions.

A comparison of sucrose and glucose in oral therapy has been completed and the resulting paper sent for publication. It was concluded that both sugars are equally effective in oral therapy.

The treatment of diarrhea by unsupervised oral therapy continues to be studied. Material now can be successfully packaged in glass bottles, and suitable orange flavoring has been developed.

Epidemiological studies continue in the Teknaf dysentery project. To obtain sex-specific attack rates, women have been employed in the field area to gather the necessary data. Data on socio-economic factors have been accumulated, but are not yet analyzed. Active bacteriological research continues, with coliform counts being done on water from ponds, tanks and wells. So far shigella species have been found only in human fecal samples. A comparative study of forms of intervention will commence early in March 1977. Three comparable study areas have been delineated, in all of which the baseline endemicity of shigellosis is now known.

Area A. In this area, an intensive health education campaign will be instituted, specially directed towards habits of defecation, particularly in children. The use of ash for hand cleansing after defecation will be promoted. There will be no other intervention.

Area B. Some tubewells exist in this area. The number will be increased to give one for each five or six families. The people will be encouraged to use this source of water only.

Area C. Tubewells will be sunk, giving one for each four or five houses. WHO-type latrines will be supplied, i.e. 15 ft. of concrete tubing with a water-seal squatting plate. The aim will be to supply at least one latrine per compound. The people will be encouraged to use the tubewells only, and will be instructed in the proper use of the latrines.

Evaluation of this comparative study, in terms of shigellosis incidence and general degree of use of facilities, will be continuous but a formal evaluation will be done in about a year's time.

A study of the effect of shigellosis on the growth of children is being carried out. Children of known age are being periodically weighed, both those who have had one or more attacks and those who have not. Preliminary results seem to indicate a long-term deficit in children who have suffered from shigellosis.

Considerable clinical research has been done on intestinal amebiasis, and incidence studies continue.

A study of the inter-relationships between nutritional status, cell-mediated immunity and infectious disease on the lines laid out in the 1976 report has been completed in the Matlab area. A paper is in the course of preparation.

With the expansion of interests of the CRL into wider fields of diarrheal disease, the Clinical Division has some fairly obvious deficiencies, of which three should have very early attention.

- (1) More clinical investigators. A gastroenterologist is an urgent requirement. A clinical nutritionist and a pediatrician should also be recruited.
- (2) Increased nursing expertise. It is suggested that suitably qualified nurses be sent for training to clinical research units overseas for periods of not less than six months. This would enable the senior nursing staff to take an important role in research within the CRL Clinical Division.
- (3) Increased ward space. The existing ward space is extremely crowded. Probably both the general ward unit and the special shigellosis unit should add more beds. Clinical research demands even more floor space.

Epidemiology Division

During the past year, epidemiologic activities have focused on the role of water in the transmission of cholera and shigellosis and, in a broader context, on the interrelation between man's environment and enteric diseases. The Committee supports this new direction, but emphasizes that the association of water or any other single environmental factor with a specific health problem is difficult because a number of variables -- cultural, educational, economic and others -- impact on the disease. As recognized by the staff, it will be increasingly important to identify these variables, and to obtain the collaboration of behavioral scientists, especially in the design of protocols.

Studies of cholera have given the CRL a broad and unique capability in cholera. As a result, expertise has been developed which is

applicable to other enteric diseases. The CRL was therefore in a good position to expand into studies of other acute enteric diseases, as it has done in recent years. Most members of the Committee felt that future infectious disease research activities should be restricted largely to the enteric diseases to exploit the CRL's unique capabilities. A minority of consultants felt that research activities, especially epidemiologic investigations, need not be so restricted if unusual study opportunities arise, provided there is full consultation with appropriate authorities and with medical institutions in Bangladesh that might have overlapping interests.

The Committee felt that it would be desirable to conduct some future field studies in geographic areas other than Matlab Thana, both urban and rural. An urban area in Dacca and one in the northern part of the country in the area near Rangpur may be particularly desirable, since these places have population and ecological features quite different from those found in current study areas.

Laboratory Division

The many investigative clinical and epidemiological projects emanating from the CRL have obtained good back-up support from the Laboratory Division. Many of the routine microbiological and biochemical tests used in large modern hospitals have been provided. These facilities are absolutely fundamental to any research and service program. For this reason they should be constantly modernized and their efficiency compared with that of similar services in other institutions. Isolation of bacteria, for example, is successful in only a portion of those cases from which one might expect positive results. This is true throughout the world, but performance at CRL should be regularly compared with that at other laboratories.

The provision of some immunological procedures should be planned on a more routine basis, and there is room for improvement in the variety of tests provided. The time has probably come to plan the establishment of a good immunology service laboratory.

Animal House

The CRL has been fortunate in recent years with having a good supply of clean, well-kept laboratory animals. The general standards in the animal house are good, and, with the exception of rabbits, the breeding supply is able to keep up with the demand.

CHOLERA VACCINE FIELD TRIALS

Dr. John Craig, Chairman of the U.S. Cholera Panel, reviewed for the members of the Committee and staff of CRL the options for efficacy trials of cholera immunogens that had been considered by the Panel. On the basis

of animal studies and tests in volunteers in the United States, it is now thought desirable to test the efficacy of a formalin inactivated, immuno-absorbed toxoid prepared by Burroughs Wellcome, and to compare this toxoid, which is made with a special aluminum hydroxide gel, with whole cell vaccine only, and with a combination of vaccine plus toxoid, the latter two preparations also to contain alumina gel. The inclusion of a Tet - Dip control group would require 100,000 subjects, or much of the accessible population in Matlab. Toxoid will have passed final testing for field use by early 1978.

Since whole cell vaccine prepared by Burroughs Wellcome has not been tested in the field, consideration had been given to a 1977 trial to compare the adjuvanted and non-adjuvanted forms of this product with the results obtained with Merck Sharp Dome whole cell vaccine in the 1960's. As previously detailed by Dr. George Curlin, such a trial would also require a large number of subjects in Matlab. Further, there are other constraints. The major one to be identified was the concern of the Bangladesh Medical Research Council (BMRC) as reported by Dr. Mjuibur Rahaman (a member of the BMRC and Acting Director of CRL) regarding the Council's responsibilities with reference to future field trials. The Committee was told that the BMRC wished for its scientists to be involved in the decision making process relative to the choice of cholera vaccines for testing and in the design and development of the protocol for a field trial. This would necessitate the participation of BMRC representatives in data review and planning, in discussions with vaccine producers and testers, etc. so that they would be thoroughly familiar with vaccine research and development before a protocol is submitted to the BMRC for its review. That these concerns were reasonable was recognized by the Committee and by CRL staff. The Director Designate then outlined several approaches to active and early participation of the BMRC and other Bangladeshi scientists. Given these concerns and those previously noted, it was agreed that there would be no field trial in 1977. The Committee felt that the question of the synergism of toxoid and whole cell vaccine is of such importance that the four-cell field trial projected for 1978 (described above) should be conducted.

This discussion highlighted an aspect of international health research which is being increasingly manifested in developing countries. Shifting attitudes regarding human experimentation combined with a growing nationalism that rejects any action that can be interpreted as the exploitation of the citizen of one country by investigators from another will demand the greatest of diplomacy and tact on the part of foreign investigators. That this is the case in Bangladesh was made clear recently in an article printed in the Bangladesh Times. The article was written by Dr. Zafarullah Chowdhury, a surgeon, who is Project Coordinator of the People's Health Center (formerly Bangladesh Hospital). Because of its relevance to the future of the CRL, a copy of the original paper submitted to the Times is included as Attachment No. 3

MEETING WITH DIRECTING COUNCIL

At its meeting with the Directing Council on the afternoon of February 25, the Committee learned with pleasure that at an Inter-ministerial meeting that morning approval had been given both for the appointment of Dr. Wiley H. Mosley as CRL Director, effective March 1, and to the proposal calling for the conversion of CRL into an International Institute. The proposal will now be forwarded to the top levels of government.

The Directing Council endorsed the trends in the CRL research program as listed in the Director's Report and as exemplified by the scientific data presented for the Committee's review. Reference was made to the apprehension that had been expressed concerning the extension of CRL research activities into the fields of population and nutrition, and to the need for coordinating work in these fields with that of the National Institute of Population Research and Training and the National Institute of Nutrition Research at Dacca University. It was agreed that duplication of effort and expense should be avoided, and acknowledged that these broad fields could absorb the talents of many investigators.

There then followed a useful discussion of vaccine field trials and of the appropriate role of the Bangladesh Medical Research Council. The Directing Council offered its support to the new Director in informing the BMRC of CRL activities, and in seeking its understanding and endorsement of the CRL research program.

COMMENTS

Recommendations regarding specific research projects have been included in the appropriate sections above. The Committee notes that many of the recommendations made in last year's report are still appropriate, and is pleased to observe that those relative to nutrition, population studies, training, and diversification of financial support have been reflected in the prospectus for the new International Institute.

The Committee again commends the CRL for its general plans for broadening its interdisciplinary activities to include a greater proportion of studies involving demography, nutrition, immunocompetence and population dynamics, particularly as these influence, and are influenced by, diarrheal disease morbidity and mortality in Bangladesh. The time is now at hand for specific and careful planning in these new areas.

While broadening the program of the CRL, however, the new Institute should not neglect the responsibilities of the Cholera Research Laboratory. The CRL has built a unique expertise and reputation in cholera studies. Surely all the problems of cholera pathophysiology and epidemiology have not been solved.

The Committee underscores its prior emphasis on the great value of the demographic data developed by the CRL. Basic demographic observations are both a logical outgrowth as well as an essential element of any field study. The critical base for any epidemiological study is establishment of the denominator, the population at risk. This inevitably requires a census to maintain an up-to-date population base. Hence registration of births, deaths, and migrations is required.

The impact of a specific disease process on the health and well-being of a population can be understood in the context of the overall level of morbidity and mortality in the population. Thus, in-depth analysis of mortality and morbidity in the population by age, sex, economic status, and other variables is required to define the critical risk factors. Disease processes, such as diarrhea, do not occur in populations as isolated phenomena. They are compounded by many factors such as malnutrition, other diseases in the populations, levels of health care available, traditional therapeutic practices, social and cultural practices relating to child care, etc. These factors, which must be understood if effective control measures are to be instituted, can be defined only by in-depth social-demographic studies. It is logical, then, as is proposed, to link research programs in enteric disease, nutrition, and population control.

Both descriptive and analytical studies of the trends and determinants of change in fertility draw in large measure on the same basic epidemiological data used to define patterns of morbidity and mortality. In considering interventions to alter fertility patterns, the CRL has established the important precedent of collaborating directly with the Family Planning Program of the Government of Bangladesh. As it must be, the Contraceptive Distribution Project is a joint CRL-GOB venture. The same is true of the Johns Hopkins Fertility Research Project which uses Matlab as a base. Clearly, the new Institute must integrate the research of this population project, just as it must integrate the diarrheal studies of the Johns Hopkins International Center for Medical Research.

With reference to the charter yet to be drafted for the proposed International Institute, the Committee expresses its conviction that the research program should continue to be reviewed by an outside, independent body. The Committee does not respond favorably to the suggestion that the Board of Directors function simultaneously as a Scientific Advisory Committee.

The Committee was pleased to learn that those planning the new International Institute are aware of the need to utilize an international scale of salaries for its scientists, regardless of national origin, as does the World Health Organization. How this will affect the support of scientists temporarily assigned from the U.S. and other countries remains to be determined.

Finally, the Committee wishes to comment on the review process per se and on the general conduct of its meeting. The Committee feels that the pressure of time experienced at this year's meeting validates the recommendations for scheduling made in last year's report. A minimum of nine days is needed to permit preparation of the final report for signature. Those who have traveled far should stay long enough to do a proper job. To make the best use of time, the Committee and its consultants should be split into groups for visits to different sites such as Matlab, Teknaf, and urban Dacca.

In this transition period, anticipated staff turnover and the need for long-range planning had an adverse effect on specific project planning. This, plus the apparent lack of coordination among projects, compromised the review process. The Committee asks that the Director and his staff consider the following:

- (1) There was a distinct tendency for the review sessions to become open seminars. This is a mistake. Meetings should be restricted to Committee members and consultants together with only those staff members who have material to present.
- (2) On a number of occasions, the standard of presentation was poor. One presentation was marred by colloquialisms totally out of place in a scientific paper. There is no excuse in a meeting of this kind for slides inserted upside down, etc. Sessions should be rigorously rehearsed.
- (3) Some of the hand-outs were marred by misspellings, mistyping, and poor English. They showed signs of very hurried preparation and some had clearly not been edited. Similarly, many slides had errors in spelling and displayed other evidence of lack of care in preparation.
- (4) There were far too many handouts, and these were distributed too late. It is totally impossible for the Committee members to read all of this material during the presentations. It should have been available to them before arrival or, at the very least, on arrival in Dacca.
- (5) The Committee spent most of its time reviewing work that had been done. It wishes to devote at least 50% of its time to consideration of proposals for new projects. Protocols of proposed projects should be available.
- (6) The time of the Committee should not be taken up by minutiae, e.g., small-scale drug trials, etc.

For the future, it is suggested that each major project be given a serial number. This will lead to better continuity from year to year. Each year those responsible for scientific review will then consider:

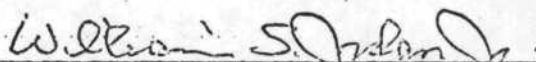
- (1) A list of projects completed, with a concluding assessment;
- (2) A list of continuing projects, with a status report;
- (3) A list of proposed new projects, with protocols.

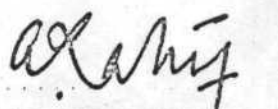
To conclude, it is noted that the word "advisory" is included in the title of the present Committee. Clearly, the review process should be an educational experience for both the members of the Committee and the staff. The yearly visit of a group of interested scientists should offer an opportunity for valuable consultation as well as for critical review. As the research programs of the CRL and the Institute are broadened and diversified, the competence of the Committee and its consultants must be expanded by those responsible for their appointment.

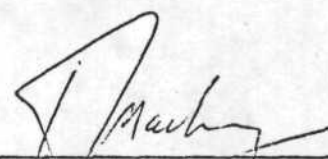
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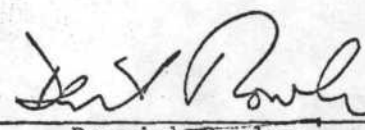
The Committee records with sadness the death during the past year of two former CRL Directors; Drs. Fred L. Soper and Robert A. Phillips.

Signed by the members of the Scientific Review and Technical Advisory Committee:


William S. Jordan, Jr., Chairman
United States Representative


M. A. Latif
Bangladesh Representative


Donald Mackay
United Kingdom Representative


Derrick Rowley
Australian Representative