CHOLERA RESEARCH
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Glimpse

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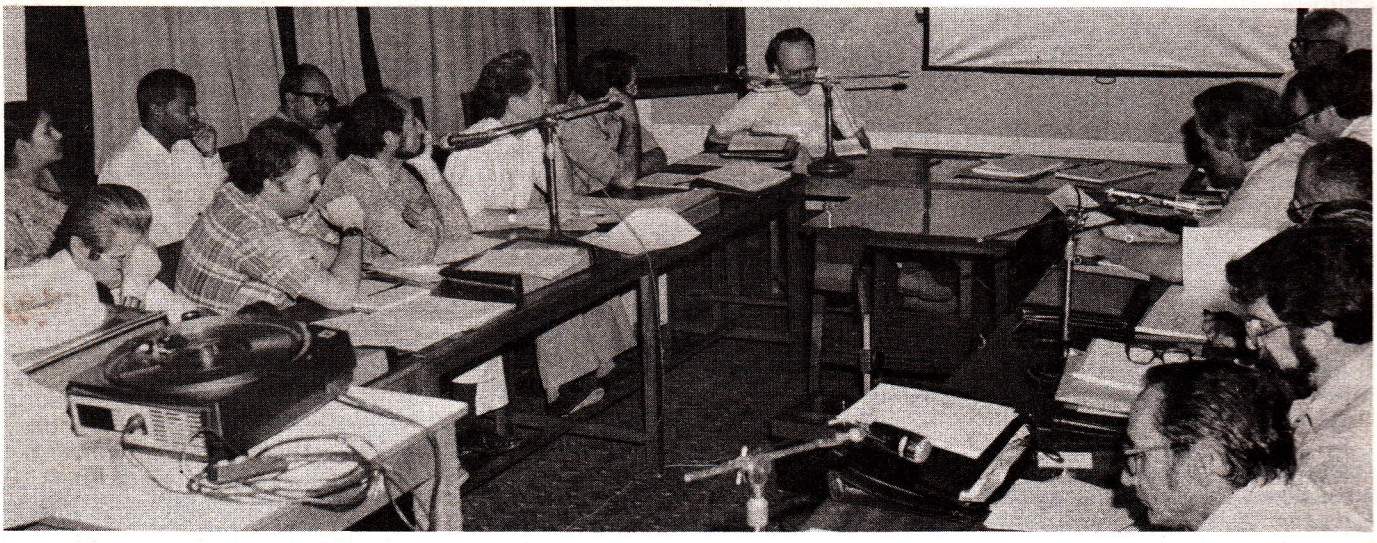

The conference on Experimental Cholera Vaccines in session.

CONFERENCE ON CHOLERA VACCINES

A Conference on Experimental Cholera Vaccines was held in Dacca, on 6-8 April 1981. The Conference was organized by the ICDDR,B in collaboration with the World Health Organization (WHO).

Following the opening ceremony, Dr. Greenough, Director, ICDDR,B introduced the objectives of the Conference:

- o to review the current status of known experimental cholera vaccines and
- o to review the process of moving the promising cholera vaccines toward a field trial.

Dr. Barua described the interest of the WHO Global Diarrhoeal Disease Program (CDD) in development of cholera vaccines. Drs. Craig, Pierce and Murphy reviewed areas of basic investigation which were relevant to the development of new cholera vaccines. Dr. Sack discussed the necessity for careful intermediate studies before proceeding to a cholera vaccine field trial. Several persons stressed the need for early standardization of any new vaccine to insure quality control and potency from lot to lot. The difficulty with some of the new vaccines is the absence of a recognized standard of potency. Development of relevant animal models should be standardized and validated to assist with potency testing.

The individual vaccines were considered. Dr. Germanier presented a new vaccine consisting of procholeragenoid combined with killed Inaba and Ogawa Vibrios to be given parenterally. The rationale for such a vaccine is that it would utilize the synergistic protection afforded by the combination of antitoxic and antibacterial antibodies.

Although the Whole Cell Vaccine (WCV) component is similar to the currently used vaccine, the toxoid antigen consists of the procholeragenoid molecule. This agregated toxin is produced by heating to 60°C for 30 minutes. Because of residual toxic activity of the progenoid after this treatment, it is further treated with formalin. Animal studies have shown immunogenicity, and protection has been demonstrated in mice and rabbits challenged with live vibrios. It has been given parenterally to volunteers in safety tests with minimal local reactions. Protection studies in volunteers have not yet been done. The vaccine has also been given to animals and

(Contd. on page 2)

Participants of the Conference on Experimental Cholera Vaccines held in Dacca from 6-8 April 1981.

- o Dr. Dhiman Barua
  Consultant to CDD
  WHO, Geneva
- o Dr. John P. Craig
  Prof. of Microbiology & Immunology
  Downstate Medical Centre
  New York, U.S.A.
- o Dr. Richard M. Finkelstein
  Professor & Chairman
  Department of Microbiology
  University of Missouri
  U.S.A.
- o Dr. Germanier
  Professor
  Swiss Serum & Vaccine Institute
  Berne
  Switzerland
- o Dr. Takeshi Honda
  Research Associate
  Osaka University
  Japan
- o Dr. Istavan Joō
  Head, WHO Collaborating Centre
  for Reference & Research on
  Bacterial Vaccines
  Szällás Utca 5. H-1107
  Budapest
  Hungary
- o Dr. Myron M. Levine
  Director
  Centre for Vaccine Development
  Maryland
  U.S.A.
- o Dr. Henry W. Mosley
  Visiting Professor
  University of Nairobi
  Kenya
- o Dr. John R. Murphy
  Associate Professor
  Harvard Medical School
  Boston, Massachusetts 02115
  U.S.A.
- o Dr. Oladeinde Ogunbi
  Professor of Microbiology
  Lagos University Teaching Hospital
  Nigeria
- o Dr. Nobuya Ohtomo
  Director of Research & Development
  The Chemo-Sero-Therapeutic
  Research Institute
  Kumamoto 860
  Japan
- o Dr. M. Ola Ojo
  Professor & Dean
  University of Ibadan
  Nigeria
- o Dr. Nathaniel Pierce
  Professor of Medicine
  Johns Hopkins University
  Maryland
  U.S.A.
- o Dr. David A. Sack
  Assistant Professor
  Johns Hopkins University
  Maryland
  U.S.A.
- o Dr. Mario Saletti
  Head, Department of Biological
  Research & Development
  Instituto Sclavo
  Italy
- o Dr. Brahm S. Srivastava
  Scientist
  Brown University
  Rhode Island, U.S.A.
- o Dr. Yoshikazu Watanabe
  Manager, Laboratory Superintendence
  Ministry of Public Health
  Kuwait

Other scientists who sent descriptions of their vaccines, but were unable to attend the conference included Drs. Agarwal, Dodin and Njoku-Obi.

CONFERENCE ON CHOLERA VACCINE

(Continued from page 1)

humans orally. There has been no evidence of reversion of the progenoid to toxicity.

Dr. Joō reviewed the information regarding the importance of adjuvant in the present WCV. Using aluminium hydroxide adjuvant, WCV gives a higher and more prolonged vibriocidal titer increase in animals. In a field trial in Indonesia involving 150,000 vaccinees protection was demonstrated. Especially significant was the 80-85% protection in children, 1 to 4 years of age, and the duration of protection extending over 1-1½ years. Aluminium adjuvants have been used for many years and are known to be safe. Other adjuvants also may become available which will further improve parenteral cholera vaccines, including liposomes and murymyl dipeptide (MDP). MDP may also be a local enteric adjuvant; however, it is very expensive at present.

Dr. Saletti presented data on two toxoid-WCV vaccines, one absorbed with aluminium hydroxide, one without the aluminium hydroxide. The toxoid is a formalinized toxoid and the WCV is a formalin killed preparation from Inaba 35A3 and Ogawa 41 grown in a fermentor. The rationale for such a WCV-toxoid parenteral combination is to stimulate both antitoxic and antibacterial antibodies which might protect synergistically. Serologic data from rabbits showed that both vibriocidal and antitoxic antibodies were stimulated by both vaccines.

Drs. Finkelstein, Honda and Levine presented information on a live mutant vaccine strain - Texas Star (streptomycin resistant) (TS-SR). The strain was derived from El Tor Ogawa 3083 (originally from Vietnam) following two episodes of mutagenesis with nitrosoguanidine. It produces B subunit but does not produce the A subunit of cholera toxin. It is intended as a live, oral vaccine which would induce immune protection in a manner similar to natural infection. Because it does not produce the A subunit however, it should not produce illness. Animal studies in chinchillas and rabbit loops have demonstrated that the vaccine does protect against challenge with both serotypes of *V.cholerae,* some LT producing *E.coli,* choleragen and LT.

The vaccine given to 42 volunteers gave an excellent rise in serum vibriocidal antibodies but an infrequent and low antitoxin response. Eight of the volunteers developed diarrhoea using the very strict criteria of the study unit, only two of these had 3 loose or watery stools in one day and the incidence of diarrhoea was not related to the dose of TS-SR.

Seven of these volunteers were 7 weeks later rechallenged with El Tor Ogawa E 7949 along with 10 non-immunized controls. Only 1 of the 7 immunized volunteers had diarrhoea and this was very mild compared to 7 of the 10 persons in the control group. No data is yet available to demonstrate heterologous or long-lasting protection in humans. Environmental studies to determine the ability of the strain to survive and compete in various environmental conditions are still lacking. In extensive testing with several animal passages, no reversions have been found.

Drs. Murphy and Ohtomo presented data on vibrio mutants induced by one of two methods: 1) temperature-induced mutation followed by nitrosoguanidine mutagenesis and 2) vibriophage-induced tox deletion. The first procedure resulted in the isolation of strains which were hypotoxigenic and had the phenotype A+B− (A reduced by a factor of 3, B reduced by a factor of 5,000 to 10,000). The genetic locus controls the trait maps in a new position on the *V.cholerae* chromosome, and differs from tox-1 and htx, ltx loci. The mutant strains are genetically stable to at least 10^{-12} by colonization studies with germ-free rats. Many such strains have been tested in animal models and found to be either avirulent or minimally virulent in these systems.

The second type of mutant (phage-induced tox deletion) utilizes a vibriophage VcAI which is thought to actually delete the tox gene and may be site specific. With DNA: DNA hybridization utilizing a cloned *E.coli* LT gene, 3 of 5 mutant strains were actually tox deletions. Other phenotypic characteristics (generation time, motility, surface antigens, prototrophy) were unchanged from the parent.

These strains are not considered at present to be vaccine strains, but are models of an approach to develop a live vaccine strain with a deletion mutant in the tox gene.

Dr. Ojo presented data on two mutant strains of *V.cholerae,* Ib5 and Ib5S. These strains were selected during a search for acid-resistant mutants and were further noted to grow better at lower temperatures (room temperature). Both grow slowly, Ib5 requires either methionine or cysteine, and Ib5S requires DNA and neither gave positive PF or ileal loop tests with culture filtrates. More sensitive tests for toxin have not been carried out.

In discussion, it was suggested that the DNA requirement may represent purine requirements. Also, the acid resistance shown by these strains might be useful in vaccine development; perhaps, the need for gastric acid neutralization could be avoided.

Dr. Srivastava presented data on two classes of live Vibrio mutants. These strains were developed in hopes of finding a vaccine strain which would possess other antigens of virulent *V.cholerae* (e.g. motility, adhesive factors, colonization, etc.) but lack the crucial ability to produce toxin. Several strains were induced which were not virulent in animal models and induced protection in rabbit loops when used as an immunogen.

Dr. Gothefors presented data on cholera B subunit. Cholera B subunit is derived from affinity column purified choleragen with subsequent purification of the B subunit. The B subunit retains the antigenic and binding properties of the holotoxin; it does not, however, have the toxic properties. Animal studies have shown it to be safe, immunogenic and protective either by parenteral or oral route. It has been given to humans by the parenteral route and oral route in Sweden and Bangladesh. Orally, there have been no side effects. Given parenterally, there were mild reactions consisting of local pain and tenderness and less commonly, induration. When given either parenterally or orally to volunteers in Bangladesh, it stimulated systemic and local intestinal antitoxin antibodies. The intestinal IgA antitoxin antibodies appeared to be longer lasting following oral immunization than parenteral. Attempts to find proxy measures of intestinal antibodies were partially successful in that saliva IgA antitoxin titer rises correlated with intestinal titer rises. When mixed with whole cell vaccine in an oral preparation, intestinal IgA antitoxin rises were uncommon after one dose, though after a second dose they were similar to rises seen in the earlier study when B subunit was given alone.

Protection studies have not been carried out in humans.

Dr. Farida Huq gave a national perspective of cholera vaccine development and said that in Bangladesh ICDDR,B was considered to be the proper place to look for a good vaccine. She elaborated on the ethical problems with field trials stating that the vaccine must pass safety tests in the country were it was developed before it is brought for further trials to a developing country. She mentioned as an example, that if the oral vaccine discussed earlier in the conference gives a mild diarrhoea for even one day, every possibility exists that it will be rejected by the community.

Dr. S Kabir presented his studies on the composition of the cell envelope of *Vibrio cholerae.* The LPS seems to have a complex branched structure and possesses similar immunobiological properties as Salmonella LPS. When the protein composition of the outer membrane was analysed all strains (two biotypes, two serotypes) contained a major protein band of molecular weight 48,000. But there were differences regarding minor proteins. Immunological tests also suggest the presence of a common protein antigen in the outer membrane of *Vibrio cholerae.* Hydrophobic forces were suggested to play a role in the process of adhesion of vibrios to the host surface.

PUBLICATIONS

1. Factors Related to acceptance of Tetanus Toxoid Immunization Among Pregnant Women in A Maternal-child Health Programme in Rural Bangladesh/*Makhlisur Rahman, Lincoln C. Chen, J. Chakraborty, Md. Yunus, A.S.G. Faruque, A.I. Chowdhury. January* 1981. *(Scientific Report No.* 43)

In a rural area of Bangladesh, as a part of a maternal-child health and family planning programme, tetanus vaccination was offered to pregnant women. During a period of 16 months, only about 34 percent of the pregnant women identified by field workers received full immunization (2 injections) and about 5 percent received partial immunization (1 injection). The major reasons reported for not accepting the vaccination were objection by husbands and mothers-in-law, fear, and failure of workers to inform pregnant women about vaccination early enough. The most frequently reported reason for failure to accept the second injection was the absence of the women from their usual residence (they preferred confinement in their parents' house).

The findings of this study did not support an apprehension that previous provision of injectable contraceptive had discouraged women from accepting tetanus vaccination. The families of tetanus vaccination acceptors appeared to accept oral therapy for diarrhoea sooner than the families of tetanus vaccination non-acceptors, suggesting that in the community some households were likely to accept a variety of modern medical technologies earlier than others. However, a comparison of acceptors and non-acceptors of vaccination showed little difference between the two groups in terms of their socio-demographic characteristics.

2. Infant Mortality in Rural Bangladesh: An Analysis of Causes During Neonatal and Postneonatal Period/*M. Shafiqul Islam, M. Mujibur Rahaman, K.M.S. Aziz, Mizanur Rahman, M. H. Munshi, Yakub Patwari. April* 1981. (*Scientific Report No.* 44)

The causes and risk factors of neonatal and postneonatal mortality were analysed by following a cohort of 1,351 infants born between July 1976 and June 1977 for a one-year period in the Teknaf Dysentery Project in rural Bangladesh. Tetanus (31%), prematurity (22%) and congenital illnesses (12%) were the commonest causes of neonatal deaths. Pneumonia (33%), malnutrition (18%), diarrhoeal illnesses (10%) and fever (9%) were the most important causes during the postneonatal period. Delivery complications of the mother and the newborn were found to be significant determinants of neonatal mortality. Infant mortality was highest for mothers below the age of 20 years and lowest between 25-29 years. The size of the family was directly related to the infant mortality rates.

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ICDDR,B
PO Box No. 128, Dacca-2
Bangladesh

COUNTRY REPORT

THAILAND

In Thailand, though diarrhoeal disease ranks fourth among the ten major causes of death, it ranks first among the causes of infant death. Based on the data available from the hospitals and treatment centres, in 1978 the morbidity rate was 3 per 1000 population, diarrhoeal diseases accounted for 22% of all hospital admissions,42% of all diarrhoeal admissions were children under 5 Case fatality among children was five times higher than young adults (2.5% as compared to 0.5%). Assuming that only 1 in 50 cases of acute diarrhoea would seek treatment from government hospitals and health centres, the actual morbidity rate should be around 150 per 1000 population.

Yearly Incidences of Diarrhoeal Diseases

Year	Number of cases reported
1974	54,486
1975	62,756
1976	73,446
1977	98,662
1978	136,759
1979	176,664
1980 (Nov)	181,938

The Inter-Regional Training Course on Diarrhoeal Diseases—Clinical Aspects was held in Dacca from 8-19 December 1980. Country reports presented by the participants are edited and summarized for our readers; this is the report presented by ***Drs. Sathaporn Manatsathit and Sawat Ramaboot*** *from Thailand.*

The steady rise in number of diarrhoea cases over the years may not indicate higher incidence, but reflect increased reporting due to increased numbers of district hospitals and rural health centres.

Intravenous fluid therapy is widely available in Thailand, even in remote areas. Prompt administration of intravenous fluid has brought down the case fatality rate from cholera and other diarrhoeal diseases in hospitalized patients to under 1.0%. However, oral rehydration therapy is not widely known among health personnel. Its use has been limited to a few hospitals and health centres. The Government Pharmaceutical Organization produces the aluminium foil package of oral rehydration salts (ORS) containing components recommended by WHO, and sold at 10 US cents. At least four hospitals prepare oral rehydration salts for their own use. Good acceptance by patients was reported from all four hospitals.

Diarrhoeal Diseases in BIDH

Bamrasnaradura Infectious Diseases Hospital (BIDH) is the major hospital for admitting all referral cases with acute severe diarrhoea, clinically suspected of having cholera, from all over the country. This objective can hardly be realised because of the vast problem of diarrhoeal diseases in the country. Practically, the majority of cases admitted in this hospital are referred cases mainly from nearby provinces around Nonthaburi, where this hospital is situated. BIDH has the capacity of 200 beds with another 200 in reserve. Stools of all acute diarrhoea cases admitted are examined and cultured. According to hospital statistics in 1979 diarrhoeal diseases account for 24.1% of the total out-patients and 51.9% of the total in-patients. The table below shows precentage of various organisms isolated from patients admitted in 1979.

Out of all positive cultures, the following were isolated:

Vibrio cholerae	33.3%
Shigella	25.7%
Vibrio parahaemolyticus	19.4%
Salmonella	13.0%
Non-Agglutinable Vibrio	6.3%
Enteropathogenic *E. coli*	2.3%

Antimicrobial Sensitivity Pattern of Pathogenic Enteric Bacteria in 1979

	Per cent Sensitivity											
	Ampicillin (10)	Cephalothin (30)	Chloramphenicol (30)	Cotrimoxazole (25)	Colistin (10)	Erythromycin (15)	Kanamycin (30)	Nalidixic a. (30)	Neomycin (30)	Nitrofurantoin (300)	Polymyxin B (300)	Tetracycline (30)
V.cholerae	99	100	99	99	0.1	100	99	99	95	100	0	99
V.parahaemolyticus	1.3	86	99	96	57	90	97	99	44	99	72	99
NAG Vibrio	66	93	96	96	39	88	97	99	63	96	50	91
Salmonella	69	96	78	76	99	—	75	99	72	90	99	71
Shigella	16	98	4	30	99	—	93	99	37	99	98	4
Enteropathogenic *E.coli*	8	63	23	51	100	—	31	100	20	77	86	17

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