news and views

Cholera and related diarrhoeas ('turista')

from Richard A. Finkelstein and Mary Boesman-Finkelstein

CHOLERA and the related diarrhoeal diseases extract an incalculable global toll in human misery, morbidity and mortality. They also have a direct impact on human nutrition and growth and affect world food supplies through their effect on livestock. On August 6–11 they were the subject of the 43rd Nobel Symposium* at which 30 experts discussed the epidemiology, molecular biology, immunology, pathophysiology and significance of the enterotoxic enteropathies as a worldwide health problem. The symposium could not have been more timely; as a result of the tremendous progress during the past decade, there was a lot to be said.

Of the many highlights of the symposium, the dramatic advances in the treatment of cholera and related diarrhoeas, the dynamic interaction between diarrhoeal disease and malnutrition, developments in understanding the pathogenesis of diarrhoea at the molecular level, and the imminence of a living attenuated vaccine for cholera and the immunologically related enterotoxic enteropathies are certainly worthy of further brief review.

The remarkable developments in therapy of cholera and related diarrhoeal diseases pre-eminently illustrate the practical rewards of basic research. The results would certainly have been gratifying to the late Dr Robert Allan Phillips who, were he alive, would have been a key figure at this conference, as it was he who pioneered both intravenous and oral fluid and electrolyte therapy. His observation (Phillips *Fed. Proc.* **23**, 705; 1964) that the incorporation of glucose in oral solutions might completely eliminate the requirement for intravenous fluids in the average cholera case was clearly substantiated in the symposium independently by L. Mata (Costa Rica), S. C. Pal (Calcutta), D. Barua (WHO, Geneva), W. B. Greenough, III (Dacca), T. Hendrix (Johns Hopkins), M. Field (Chicago), and M. Levine (Maryland). All but a small percentage of diarrhoeal patients can now be treated successfully solely with orally administered electrolyte solutions. This can be accomplished, effectively and economically, by the distribution of packets containing preweighed amounts of glucose, NaCl, KCl and $NaHCO_3$ to be added to measured volumes of water in the hospital, in local treatment centres and, most importantly, in the home, even in the most rural and underdeveloped areas. In a small number of severe cases, particularly those with emesis precluding oral rehydration, intravenous fluid therapy is still required, but even some of these can be treated orally if the electrolyte solutions are administered in small but frequent increments. It is only a minor limitation that antibiotics, which have been shown to reduce the duration of bacterial shedding and of the diarrhoea, are not presently included. The impact that decentralisation of treatment might have on the ecology of the pathogens involved should be a subject of surveillance in the future. Other questions that remain to be answered are whether other energy sources can be substituted for glucose and whether different formulae are required for diarrhoea due to rotaviruses or other agents.

That certain antibiotics, administered prophylactically, can reduce the incidence of diarrhoea in travellers was established by B. Sack (Johns Hopkins University) in studies with Peace Corps volunteers. In this population, enterotoxigenic *Escherichia coli* are responsible for the majority of cases and, fortuitously at present, the majority of these strains are antibiotic sensitive. However, because of the spectres of drug resistance and alterations of normal flora leading to superinfection, participants at the conference agreed that prophylactic antibiotics, if used at all, should be restricted to short-term travellers at high risk.

The possibility of pharmacological intervention after the onset of diarrhoea was also raised and supported by experimental studies by Hendrix and I. Lönnroth (Göteborg). At the moment, two drugs, chlorpromazine and nicotinic acid, show promise in animal studies and merit further consideration for controlled study in man.

Contrary to popular belief, evidence presented by Mata indicates that rather than malnutrition predisposing to infection, the reverse seems to be the rule—'malnutrition is determined by diarrhoea.' With newborn infants and children in particular, especially in developing areas, there is a tendency to withhold nutrients during diarrhoeal illness. Mata's studies in Central America, supported by observations of Barua, clearly indicate that when such children are adequately treated, by oral therapy, during their multiple bouts of diarrhoea, they grow better than their counterparts — the stunting which occurs during and following illness does not happen. This work has the important implication that availability of food is not the major problem in 'malnourished' children. Rather, the problem is to educate the people to provide proper treatment and normal (adequate) diet throughout periods of diarrhoeal disease. This is particularly important during the nursing period. Mata's message was also that we should be careful to differentiate between susceptibility and severity, recognising that the malnourished individuals are most frequently found in poor sanitary environments.

Epidemiologically, it was acknowledged that cholera declines when the water supply is freed of contamination with human faeces. It is not clear, however, that this is the case with other enterotoxic enteropathies such as *E. coli* diarrhoea, which seems to be transmitted primarily within the household and by food. The problems of developments in sanitation, which could solve the cholera problem, while recognised by the participants, were felt to be beyond the province of this conference.

Presentations by M. Gill (Harvard University) and M. Field (University of Chicago) revealed that for the second and third times (diphtheria being the first), the pathogenesis of infectious diseases is within a hair's breadth of being totally understood at the molecular level. Field, who inspired the recognition that cholera enterotoxin (choleragen) acts by activating host cell

*The symposium was held at the Nordic Education Centre, Stockholm, and was excellently organised by Örjan Ouchterlony, Jan Holmgren, John Craig and Dhiman Barua. Review papers provided the foundations for workshops culminating in recommendations for the future. The complete proceedings, workshop summaries and recommendations are to be published by S. Karger AG, Basel, early in 1979.

Richard A. Finkelstein is Professor of Microbiology and Mary Boesman-Finkelstein is Faculty Associate in Biochemistry, University of Texas Southwestern Medical School at Dallas.

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adenylate cyclase, presented new evidence that ST (the heat-stable low molecular weight enterotoxin(s) of *E. coli*, of which one example was characterised by D. Robertson (University of Kansas) as a peptide of 47 residues) acts by activating host cell (specifically intestinal epithelium) guanylate cyclase, while Gill indicated that the A-subunit of choleragen catalyses primarily the ADP-ribosylation of a GTP-binding protein which, according to observations by Z. Selinger, traps adenylate cyclase in its activated state. The subsequent events, catalysed by cyclicAMP or cyclicGMP, which lead to the increased electrolyte and fluid secretion, are still a grey area.

S. Falkow (University of Washington, Seattle) dynamically demonstrated the application of the newly-developing recombinant DNA technology to the solution of problems of pathogenesis, with the object of creating useful new microbial strains, rather than new pathogens, with evidence for the cloning of the ST and LT (heat-labile cholera-related enterotoxin) genes of *E. coli*. He supported the observations of F. Ørskov (University of Copenhagen) that the *E. coli* strains which are selected to become enteropathogens (that is, which can effectively receive *tox* plasmids and other essential virulence factors) may be restricted to a relatively small number of serotypes which happen to be relatively good genetic recipients. Some 'non-toxigenic' strains of *E. coli* of proven pathogenicity for man in volunteer studies may produce toxins which are active in other than the usual models, according to Levine. Other essential attributes of virulence, such as chemotaxis and adhesiveness leading to colonisation, were discussed by R. Freter (Michigan). J. Craig (New York) pointed out that cholera vibrios differ in the amount of toxin they elaborate *in vitro* under two different conditions of cultivation. The advantages and limitations of available techniques of recognising enterotoxins and their antibodies were discussed by Ö. Ouchterlony, who also presented a novel simple method of assay based upon changes in hydrophilic character of antigen-antibody complexes on plastic surfaces. The essential roles of the host cell glycolipid receptors, the G_{M1} ganglioside, elucidated by the pioneering observations of W. E. van Heyningen, were reviewed and extended by L. Svennerholm and J. Holmgren (Göteborg).

The possibilities of immunological intervention were high among the priorities of the Symposium. J. Feeley (Atlanta) extensively reviewed the scientifically controlled field studies of cholera vaccines composed of killed vibrios administered parenterally, and concluded, in accord with previous judgment, that the benefits derived did not justify the cost of vaccination. According to Barua, in consideration of these observations, requirements for cholera vaccination were removed from WHO regulations in 1973. Nevertheless there is the possibility of improvement, as brought out by more recent studies, presented by I. Joó (Hungary) and Pal, on alum-adjuvanted bacterial vaccines which seem to generate greater protection in the susceptible infants and children of endemic areas than previously tested un-adjuvanted vaccines. Although cholera toxoid administered parenterally offers no protection according to the field studies summarised by N. Ohtomo (Japan) and G. Curlin (Dacca), the synergistic protective effect observed experimentally with combinations of toxin antigen and somatic antigens in studies by A.-M. Svennerholm (Göteborg) is worth further consideration. She also indicated the potential value of assaying the antibody content of milk from immunised lactating women as a reflection of the local (intestinal) secretory antibody response. N. Pierce (Johns Hopkins University) reviewed extensively his observations on the kinetics of formation of antibody-containing cells in the lamina propria in response to a variety of combinations of orally and parenterally administered toxin antigens in experimental animal models and concluded that the nature of the antigen is important and that immunological suppression may result from some regimens. J. Holmgren (Göteborg) indicated that species differences may also be important, as illustrated by his studies in the orally immunised mouse model described earlier by Fujita and Finkelstein. J. Murphy (Harvard) reviewed genetic studies in his laboratory which have resulted so far in the isolation of two hypotoxinogenic mutants of strain 569B which produce reduced amounts of subunit A and little, if any, subunit B.

That solid immunity against cholera is indeed feasible has been established conclusively by studies in volunteers as summarised by Levine. Volunteers convalescent from induced cholera were found to be virtually completely refractory to subsequent challenge with cholera vibrios of either the homologous or heterologous serotype. Therefore it is clear that the disease itself is an immunising process, although the mechanism(s) and the nature of the immunising antigen(s)—somatic antigens, enterotoxin, colonisation factors, flagella, outer membrane proteins, or a combination—are not yet known. Immunity to rechallenge after induced *E. coli* diarrhoea was markedly less effective.

In an earlier session, R. A. Finkelstein (University of Texas, Dallas) summarised the laboratory production and isolation of enterotoxins; elucidated criteria for defining enterotoxins and 'enterocytotoxins'; issued a caveat against the tendency to regard all newly described toxicities as toxins; and also reported the isolation with T. Honda, of a mutant of *Vibrio cholerae* which offers promise as a live vaccine against cholera and related diarrhoeas. Discussed further in the Immunology workshop, the mutant, called 'Texas Star', is avirulent in the sensitive infant rabbit model, has shown no signs of reversion, and seems to produce only the immunogenic B (binding) portion of the cholera enterotoxin. It is hoped that the mutant will be shown to be innoccuous in volunteer studies and that it will simulate infection with virulent vibrios by producing solid immunity to cholera, and potentially to other diarrhoeas as well, by means of the common toxin B antigen. As a previous hypotoxinogenic mutant, M13, isolated in our laboratory, was harmless in volunteers and induced cholera immunity, this expectation may not be unrealistic.

In the immunology workshop it was emphasised that in volunter studies, which are an effective step toward vaccine development, the highest internationally acceptable ethical standards must be followed with regard to selection, education, and respect for the rights of volunteers.

Participants were reminded of how far we have come in a short time by the presence of one of the Indian investigators, S. N. De (Calcutta), who in the late 1950s first showed that the symptoms of cholera could be produced in laboratory models by cell-free products of the cholera vibrio. □

Ultra-cold neutrons in superfluid helium

from P. V. E. McClintock

THE first measurements of the rate at which ultra-cold neutrons are produced within a vessel of liquid helium, reported in a recent issue of Physics Letters (**66A**, 469; 1978), strongly support the earlier suggestion by Golub and Pendlebury (Physics Letters **62A**, 337; 1977) that it might be possible to fill a neutron bottle with trapped neutrons to an unprecedentedly high density if superfluid ^{4}He were used as the internal medium. The production measurements were carried out in Grenoble at the Institut Laue-Langevin by P. Ageron and W. Mampe (ILL) in collaboration with R. Golub and J. M. Pendlebury (Sussex University).

Ultra-cold neutrons (UCN) are neu-

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