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PRINCIPLES AND PRACTICE OF CHOLERA CONTROL

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PUBLIC HEALTH PAPERS
No. 40

PRINCIPLES AND PRACTICE OF CHOLERA CONTROL

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WORLD HEALTH ORGANIZATION

GENEVA

1970

PRINTED IN FRANCE

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PREFACE

Although cholera endemic areas are geographically limited, they contain a large part of the world's population. Moreover, modern means of travel permit an even more rapid spread of the infection than in the past, and the existing International Sanitary Regulations, as at present applied, have proved unable to stop the advance of the seventh pandemic. Its extension during 1970 is a clear reminder that cholera continues to be an international problem. The spread of cholera can, in fact, only be prevented by continuous surveillance and by a full and free exchange of epidemiological information on an international level. It must be recognized that there is no longer any need for cholera to be thought of as a dreaded disease.

Knowledge of cholera has expanded to a remarkable degree during the last decade. The development of more effective therapy, a fuller understanding of the pathogenesis and pathophysiology of the disease, the exploitation of animal models for the study of immunity, the critical assessment of the value of the vaccines at present available, and the demonstration of the prevalence and importance of the carrier state are highlights of the progress made. Since much of the information is available only in scattered sources, the present compilation has been undertaken under the auspices of WHO. It is not intended to be exhaustive; those who require more detailed information are referred to the already published literature. Most of the chapters have been adapted from papers originally prepared for a WHO document "Cholera: a review for WHO seminars". Others have been written especially for the present purpose.

For quick and easy reference a concise review of the subject, summarizing present knowledge and outlining practical measures for cholera control, has also been prepared. This review, which will be found in the Supplement on pages 123-139, is intended especially for those areas where cholera has been unknown for many decades and where the approach or the appearance of the disease would confront the health authorities with unfamiliar problems. Further advice will, of course, be gladly supplied by WHO on request.

CHAPTER 1

CHOLERA AS AN INTERNATIONAL HEALTH PROBLEM

B. CVJETANOVIĆ[a]

INTRODUCTION

Cholera has played an important, if not decisive, role in the development of concepts of international health. The rapid increase in traffic and trade in the last century led the major powers of that time to meet (in 1851) and to agree upon the "International Sanitary Convention", not only in order to protect themselves against cholera, a "disease of fear", but also to avoid incalculable and needless losses in trade that could result from this disease. Indeed, the foundation of international health organizations such as the *Office international d'Hygiène publique*, the Health Section of the League of Nations, and the World Health Organization was due to the ever more pressing international health problems caused, to a large extent, by cholera.

The pandemics of cholera that ravaged the world in the nineteenth century were so violent that the old fears are by no means forgotten. Even though the present pandemic—the seventh—is much less serious than those of the past, the word cholera still has the connotation of disastrous epidemics and creates panic even among sections of the medical profession. This has led to exaggerated reactions in some countries, and cholera, essentially a public health problem, has become an international economic problem as well.

The health problem is real but easily dealt with, whereas the economic problem is elusive and difficult to resolve.

EXTENT OF THE PROBLEM

Although cholera is endemic only in Asian countries, it should be borne in mind that:

(1) more than half the total world population lives in or near the endemic areas, which contain enormous cities and agglomerations;

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(2) with present-day modes of travel, cholera can spread rapidly from the endemic areas to many other parts of the world.

In terms of the population potentially at risk, therefore, the problem is much greater than appears at first sight.

It should also be noted that Asia is among the least developed areas of the world and very few Asian countries have any prospect of eradicating cholera while the population explosion and the slow pace of development continue to foster poverty, ignorance and poor sanitation, the conditions on which cholera thrives.

The problem is therefore international from two points of view. Firstly, although cholera is of direct concern only to the countries where outbreaks have occurred, it nevertheless threatens to affect neighbouring countries and the rest of the world. Secondly, neither the immediate task of cholera control nor the long-term project of eliminating the endemic foci can succeed without international co-operation and assistance.

APPROACHES TO THE PROBLEM

Approaches to international action have varied over the centuries, and in this rapidly changing world it is clear that concepts and strategies need to be revised yet again.

The principle of the *cordon sanitaire*, with armed guards, quarantine stations on isolated islands, fumigation and burning of infected houses, and the like, is no longer valid, for it is effective in preventing the importation of disease only when there is complete severance of all contacts such as travel and trade with any country where cholera exists. The impracticability of such a policy in modern times is obvious.

The International Sanitary Regulations[1] currently in force aim at combining a maximum of safety with a minimum of interference in international traffic and trade. Notification, if carried out properly, helps adjoining territories to prepare for an emergency. The requirement of a vaccination certificate at frontiers may not help significantly to prevent the spread of cholera, but vaccination affords some degree of protection to travellers entering a cholera-affected territory. The Regulations also provide for certain measures to be adopted by infected countries to prevent the exportation of disease, but these are seldom applied despite the fact that none of them should affect international traffic and trade. The problem, therefore, is not the application of the International Sanitary Regulations, but the imposition of measures in excess of the Regulations.

[1] Revised Regulations, to be known as *International Health Regulations*, will come into force on 1 January 1971 (see Annex to Chapter 15).

Measures such as restrictions on the importation of various harmless foods cannot be justified. Sooner or later such measures, aimed at "protecting" the country against cholera, bring about considerable reductions in international traffic and trade, and the countries that suffer from such action are often ready to retaliate. The end result is often a deterioration in international relations and great economic loss, while cholera continues to spread freely.

There is little hope of developing effective international co-operation in the control of cholera unless the ideas leading to *cordon sanitaire* policies are abandoned. International co-operation can only be based on the application of scientific data and up-to-date knowledge of cholera.

Briefly, the main facts to be borne in mind when planning an international cholera control programme are as follows:

1. Immunization is effective only partially and for a short period, irrespective of the number of doses given to the population in endemic areas.

2. Not all carriers can be detected or treated with certainty. Thus, neither immunization (often carried to excess) nor examination for the carrier state is entirely effective in preventing the spread of cholera.

3. The viability of vibrios in foodstuffs is very limited, except in the case of milk and milk products; restrictions on imported food and other goods are therefore unwarranted.

4. Sanitation is effective; a country with good basic health services and high standards of personal and community hygiene can be considered as non-receptive.

A realistic international programme for cholera control should be based on:

1. Application of only those measures permitted by the International Sanitary Regulations, in order to maintain international traffic and trade and facilitate co-operation.

2. Application of adequate sanitary measures (food control, excreta disposal, safe water supply) supported by proper health education in all areas, particularly where there is international traffic (e.g., airports, seaports, roads, railways, fairs, pilgrimage routes).

3. Surveillance of cholera on a national basis and exchange of information with other countries, so that governments are aware of any possible danger of spread and can take appropriate measures at vulnerable points.

Continuous surveillance of enteric infections and cholera will enable health authorities to foresee and forestall any danger. If information so collected is regularly and freely passed on to other countries, this will engender trust and respect. The concealment of facts about cholera

—which in any case is rarely successful in these days of extensive travel—creates distrust and tends to provoke excessive precautionary measures. It is interesting to note that inordinate restrictions have rarely been imposed exclusively against India, which regularly reports cholera and leaves no scope for suspicions.

Bilateral or multilateral international co-operation in surveillance and control opens the way to mutual understanding, negotiations and assistance in solving the problems posed by the international spread of cholera. Close co-operation of this kind will enable countries to find ways and means of controlling cholera without imposing excessive restrictions on trade and travel. When full information is available it will often be found that the danger is not widespread, but is limited to a certain border area or population.

A change of attitude is needed in many countries. Members of the medical profession, with their ability to understand the biological aspects of the problem, should present the full facts about cholera to the administrators, who often have no medical training but have to decide what measures to take.

NEW PROSPECTS

Experience gained from the application of excessive measures in the western Pacific area in the early days of the present pandemic has led to the development of bilateral and multilateral co-operation in the control of cholera in that area. The fact that cholera El Tor is endemic in the Philippines is no longer a cause for disruption of traffic and trade between that country and Japan. The programme for the control and eradication of cholera in the Philippines is being directly assisted by the Japanese Government and is bringing immense benefits to both countries with the development of tourism, trade and international co-operation in that part of the world. Instead of being a cause of suspicion and tension, as is the case in some other areas, cholera has led to greater trust and improving co-operation. This is only one of a number of examples of a trend that deserves to be followed.

However the cholera situation may develop in the immediate future, the following points should be borne in mind :

1. Treatment of cholera is now so effective that nobody should die of the disease if diagnostic and treatment facilities are readily available. Countries that have such resources have no reason for panic, even when invaded by cholera.

2. Cholera can now be dealt with effectively on an international basis without any restrictions on trade and traffic.

3. The success of international action depends on the removal of false notions about cholera among the population and in health administrations.

CHAPTER 2

CHOLERA DURING THE PERIOD 1961-1970

D. BARUA[a] & B. CVJETANOVIĆ[b]

At the end of the sixth pandemic, around 1923, cholera retreated to its homeland in the deltas of the Ganges and Brahmaputra Rivers. There its incidence gradually declined even in endemic foci, although from time to time, particularly during fairs and festivals, the disease reached epidemic proportions and invaded neighbouring territories. Reports of outbreaks of cholera during the Second World War in areas like the Ukraine, completely isolated at that time from the known sndemic foci, are evidence of the capricious nature of this ancient ecourge (Friza & Rotter, 1966).

An endemic focus of a cholera-like disease caused by El Tor vibrios was discovered in 1937-1938 on the island of Sulawesi (Celebes) in what is now Indonesia (de Moor, 1939). This disease, which became known as paracholera, showed no epidemic tendencies, although it occasionally spread to Djakarta and Singapore (Felsenfeld, 1963). In 1961, however, paracholera became non-seasonal in Sulawesi and by May/June had invaded several other islands of the archipelago; this was believed to be due to the shifting of the Chinese population and to troop movements. Sarawak became infected in late June following a regatta. Rumours of outbreaks in Kwangtung on the Chinese mainland remained unconfirmed, but the disease continued its onward march, reaching Macao and Hong Kong in August and the Philippines in September. It will probably never be known how this seventh pandemic actually began and how the disease was introduced into the various countries.

The outbreaks were completely unexpected. The acute lack of experience of cholera in the countries affected, due to the long absence of the disease and the retirement or emigration of trained personnel, was the cause of much delay in diagnosis and treatment and in the planning of effective control programmes. It was a long time before

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it was realized that the offending organism was El Tor vibrio from Sulawesi and not classical *Vibrio cholerae* from the Indo-Pakistan subcontinent, although this was of limited practical importance.

In view of the changing epidemiology of El Tor infection, WHO convened an emergency meeting in May 1962 of the Committee on International Quarantine, which recommended that cholera due to El Tor vibrio be treated in the same manner as cholera due to classical *V. cholerae.*

It is difficult to give an accurate account of the cholera situation in the world to-day because surveillance is not easy. The disease occurs mainly in developing countries where basic health and laboratory services are inadequate for the detection and reporting of all cases. Although the diagnosis of overt cases is simple, cholera can mimic any enteric infection with a wide range of clinical severity. It also exhibits the "iceberg phenomenon", asymptomatic vibrio infection being much more frequent than clinical disease.

Although all cholera-affected countries undertake some sort of surveillance, there is no uniform system either of surveillance or of reporting cholera cases to WHO. Notification may be impeded by lack of resources or by an unscientific attitude towards the problem of cholera. Most governments report the number of clinical cases of cholera detected or admitted to hospital, but some report only the bacteriologically confirmed cases. Nevertheless, it is possible to follow the trend of spread of the disease from figures reported to WHO.

By 1965, classical *V. cholerae* had been almost completely replaced by El Tor vibrio in India. In East Pakistan, however, the classical *V. cholerae* serotype Inaba is still predominant; it caused a severe epidemic in West Pakistan in 1968 and isolations have again been reported from some cases in West Bengal, India. The spread of cholera during the period 1961-1970 is illustrated in Fig. 1.

In 1969, cholera was more widespread than in the preceding three years. Laos reported cholera for the first time during this pandemic. Hong Kong and the Republic of Korea were affected again.

Despite the suppression of information by certain countries, 1970 has proved the worst year of this pandemic so far, although the disease may extend even further in the next few years. During 1970 cholera did not create much of a problem in eastern Asia, except that Indonesia and Korea had relatively big outbreaks and the disease reappeared in Sabah, Sarawak, and Brunei, where it had not been recorded since 1964-65. Around the eastern Mediterranean, however, cholera became very widespread and its most significant excursion was to both Asian and European areas of the USSR and to countries in North and West Africa (Fig. 1). During the last decade cholera has been reported from

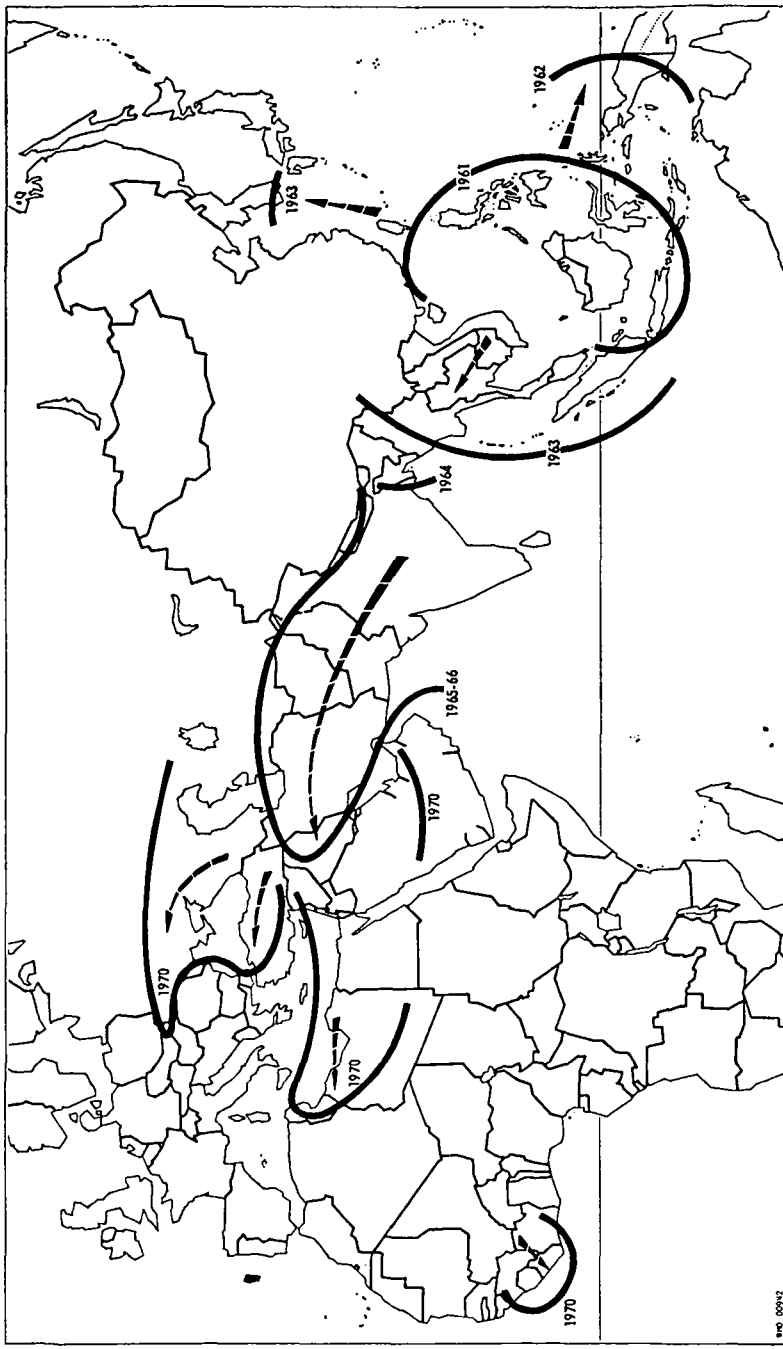
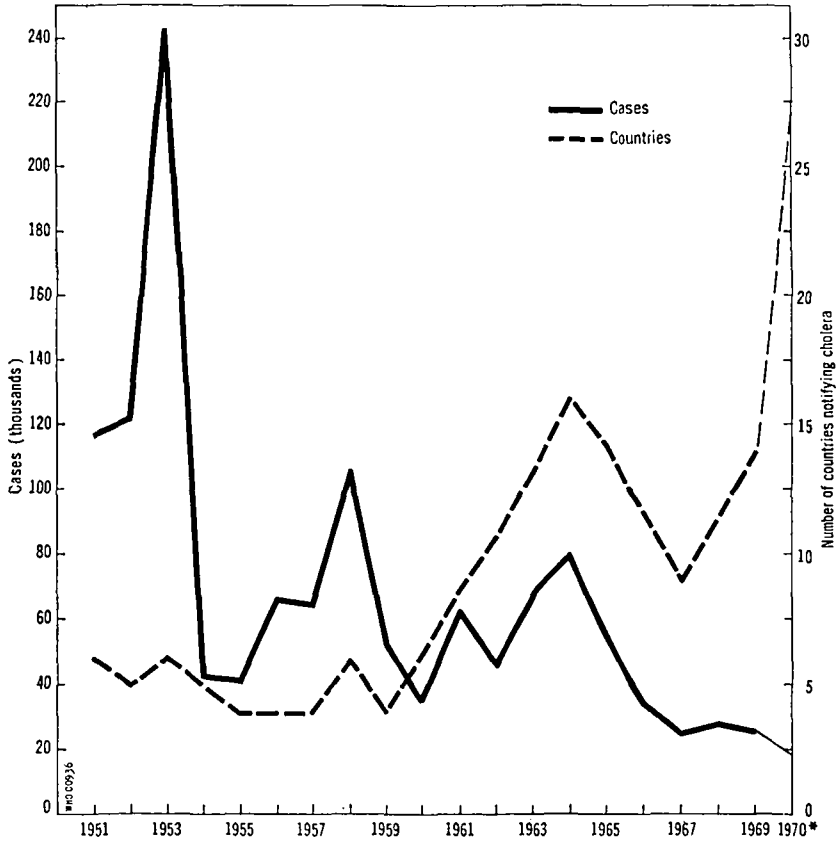


FIG. 1. EXTENSION OF CHOLERA 1961-1970

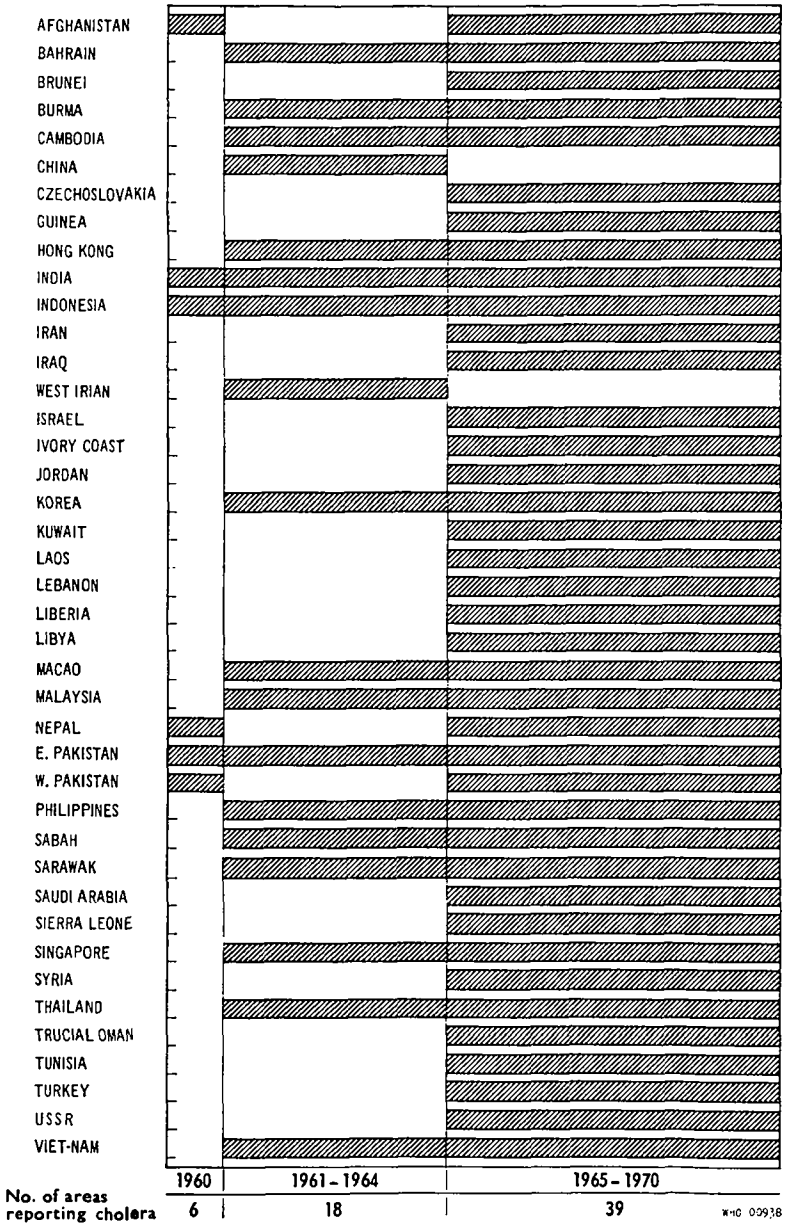
at least 40 territories: Afghanistan, Bahrain, Brunei, Burma, Cambodia, China (Taiwan), Czechoslovakia, Guinea, Hong Kong, India, Indonesia (including West Irian), Iran, Iraq, Israel, Ivory Coast, Jordan, Kuweit, Laos, Lebanon, Liberia, Libya, Macao, Malaysia (Malaya, Sabah, Sarawak), Nepal, Pakistan (East and West), Philippines, the Republic of Korea, the Republic of Viet Nam, Saudi Arabia, Sierra Leona, Singapore, Syria, Thailand, Trucial Oman, Tunisia, Turkey and the USSR (Astrakhan, Odessa, Kerch, Uzbekistan). Some of these countries have been infected repeatedly after remaining free of cholera for some years, and several have become truly endemic.

FIG. 2. INCIDENCE OF CHOLERA AND NUMBER OF COUNTRIES NOTIFYING CHOLERA, 1951-1970



Figures acc. to World Health Statistics (ex-Epidemiological & Vital Statistics) Reports
*Provisional figures (until 30 October)

FIG. 3. NOTIFICATIONS OF CHOLERA BY DIFFERENT AREAS IN 1960, 1961-1964 AND 1965-1970



The yearly incidence of cholera since 1951, according to figures reported to WHO, is shown in Fig. 2. The decline that began in 1954 has been interrupted only twice during the present pandemic: in 1958 and 1964. Since 1965, there has been a marked decrease in the number of cases reported, but at the same time there has been a considerable expansion of the area affected by cholera. Before 1961, cholera was reported annually from 3-6 territories of Asia; since then, it has been reported each year from 9-27 countries in three continents (Fig. 3). In India, only 6-10 different states used to report cholera, but since invasion by El Tor in 1964 17 states have reported it; prior to this some of them had been free from cholera for a long period, or had never known the disease.

During the present pandemic, cholera has been introduced into Japan several times by sea and by air but failed to gain a foothold owing to the efficiency of the basic health services and their surveillance activities. Cases imported into Australia, Ghana, the Republic of China and the United Kingdom were detected and dealt with equally promptly.

The outbreaks of cholera-like disease in Kumbhmela in India (Gupta et al., 1956), in Nitra in Czechoslovakia (Aldova et al., 1968) and in El Gedaref in the Sudan (*Wkly epidem. Rec.*, 1969) caused by the "non-agglutinable" or "non-cholera" vibrios indicated that these organisms should also be considered as potential pathogens able to cause symptoms similar to those of cholera under certain conditions. So far, these organisms have not shown much epidemic tendency.

Several careful studies have shown that the El Tor biotype can cause severe manifestations of cholera indistinguishable from those caused by classical *V. cholerae* and that it is more resistant to various environmental factors and antibiotics. El Tor vibrio survives a little longer in the environment, but not for an epidemiologically significant period. It causes more mild and asymptomatic infection than the classical vibrio, and fewer secondary cases in families. A few chronic carriers of El Tor cholera have also been found.

The above observations bring out certain similarities between cholera due to El Tor vibrio and the disease caused by *Shigella*. The replacement of classical *V. cholerae* by the more resistant El Tor biotype as the predominating etiological agent of cholera at the present time resembles the replacement of delicate *Shigella* species by the more hardy *Sh. sonnei*. However, the unpredictable ability to spread under suitable circumstances and to cause death in a few hours if not promptly treated emphasizes the greater seriousness of cholera and the need for increased vigilance.

REFERENCES

Aldova, E. et al. (1968) *J. infect. Dis.*, **118**, 25-31
Felsenfeld, O. (1963) *Bull. Wld Hlth Org.*, **28**, 289-296
Friza, F. & Rotter, K. (1966) *Z. Tropenmed. Parasit.*, **17**, 3-5
Gupta, N. P. et al. (1956) *Ind. J. med. Sci.*, **10**, 781
Moor, C. E. de (1939) *Mededeelingen van den Dienst der Volksgezondheid in Ned.-Indië*, **28**, 320-355
Wkly epidem. Rec., 1969, **44**, 10

CHAPTER 3

EPIDEMIOLOGY OF CHOLERA

W. H. MOSLEY[a]

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Cholera has been endemic in the delta of the Ganges and Brahmaputra in eastern India and East Pakistan since the beginning of recorded history. Between 1817 and 1923 cholera extended beyond this area in six pandemics, spreading over most of the globe along the trade routes. After 1923, cholera was again relatively confined to the endemic regions and adjacent countries of South-east Asia, except for one isolated epidemic in Egypt in 1947. The most recent pandemic, the seventh, spread from a focus in Indonesia in 1961, and by 1963 had extended northward as far as Korea, China (Taiwan), and the Philippines. The westward spread began in 1964, and by 1966 had encompassed West Pakistan, Afghanistan, Iran, the southern USSR and Iraq.

EL TOR CHOLERA AND CLASSICAL (ASIATIC) CHOLERA

Following the discovery of the cholera vibrio by Robert Koch in 1884, it was observed that the wide variety of vibrios commonly found in nature were characteristically haemolytic whereas true cholera vibrios were not haemolytic. Consequently Kraus, in 1903, suggested the use of a test for haemolysis as a means of distinguishing between non-pathogenic vibrios and pathogenic cholera vibrios. This distinction seemed valid until 1906, when Gotschlich isolated haemolytic strains of cholera vibrios from the dead bodies of pilgrims seen at the El Tor quarantine station in Egypt. Since there was no cholera epidemic at that time, the pathogenicity of haemolytic (El Tor) cholera vibrios was not ascertained. In 1939, de Moor described endemic cholera in

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Sulawesi (Celebes), Indonesia, that was due to the haemolytic (El Tor) biotype of *Vibrio cholerae*, and this El Tor vibrio has subsequently become the etiological agent in the seventh pandemic. Interestingly, the El Tor biotype of *V. cholerae* has lost its haemolytic characteristic in recent years and is now distinguished from the classical vibrio by resistance to Mukerjee's phage IV, resistance to polymixin, and the ability to agglutinate chicken red blood cells.

There are marked epidemiological differences between El Tor and classical cholera. The infection-to-case ratio is higher with El Tor cholera, and the El Tor vibrio is generally hardier, surviving longer in the environment, which makes it more easily detectable in bacteriological surveys of water and night-soil samples. The few chronic carriers of *V. cholerae* described in the literature have all been infected with the El Tor biotype.

SEASONAL PATTERN

Like other infectious diseases, cholera has a characteristic seasonal pattern, although the season varies from place to place. For example, in Dacca, the cholera season follows the monsoon rains and the disease usually disappears during the hot, dry months. By contrast, in Calcutta, at the other side of the Ganges-Brahmaputra delta, the cholera season characteristically rises to its peak during the hot, dry season and ends with the onset of the monsoon. In some areas of the Philippines, cholera tends to reach its peak during the rainy season. The cause of the seasonal pattern of cholera is unknown.

PATTERN OF SPREAD

There are two types of epidemic, explosive and protracted. In an explosive epidemic, there is a common source or a common vehicle and a large number of cases appear in a community over a short period of time (1-5 days), making it easily identified. Classic examples of common source outbreaks are the Broad Street pump epidemic described by John Snow in London in 1854, caused by contaminated water, and the explosive epidemic in Negros Occidental (Philippines) in 1962 due to the ingestion of contaminated, fresh, raw shrimps.

Cholera epidemics sometimes follow a protracted pattern, with only a few cases per day or week over several weeks. In these outbreaks the means of transmission is not always well defined. In East Pakistan, such epidemics have been primarily related to transmission by water.

Usually a large body of water, such as a river, tank or canal, becomes contaminated and the community is exposed to a relatively low dose of *V. cholerae*. Although ultimately a high proportion of the community may be infected, only sporadic clinical cases may appear. Careful investigation often reveals numerous inapparent infections and occasional small, explosive outbreaks in family groups that have a common food and water supply.

The protracted pattern may also be due to contact spread, but this has not been conclusively documented. Cholera is not easily spread by person-to-person contact. Repeated bacteriological examinations of hospital attendants of cholera patients and of neighbourhood contacts not sharing a common water supply have rarely revealed infection, suggesting that person-to-person spread by contact is very rare. Bad environmental sanitation, particularly a lack of adequate supplies of fresh water for all personal uses, seems to be the fundamental factor in the spread of cholera. Transmission is maintained in a cycle involving the vibrio excretor and the environment, and sources of water play a most important role. Provision of abundant amounts of pure water for all personal uses can break this cycle, and if this is achieved cholera will disappear as it already has from all the developed areas of the world.

SUSCEPTIBILITY

Cholera usually affects individuals of the lower socio-economic groups because of their poorer sanitary discipline. There are no recognized nutritional or gastro-intestinal factors that predispose to infection or illness. Sex is of some significance in that when an epidemic strikes a new area the earliest cases are often predominantly adult males; this is apparently due to the greater mobility of the male population, which leads to their higher exposure to potential sources of infection. Once the disease is entrenched in a community, the case rate is equal in both sexes. The age pattern varies between epidemic and endemic cholera. When a cholera epidemic spreads to previously unaffected areas, the disease characteristically affects adults. In the endemic cholera areas, on the other hand, the attack rate is distinctly higher for children than for adults, because people in these areas naturally acquire immunity as they grow older.

INCUBATION PERIOD

The incubation period of cholera is variable, generally ranging from 1 to 5 days. In one common-source outbreak, the median incubation period was 48 hours.

RESERVOIR OF INFECTION

The only known natural reservoir is man. Cholera is maintained by a cycle of transmission from man to man through the environment. Although an infected individual will usually excrete vibrios for only a few days, the high rate of inapparent infection permits this cycle of transmission to be maintained. Maintenance of infection in the endemic areas is also facilitated by the relatively brief immunity that develops following infection, so that reinfections from year to year are common. A few chronic carriers (persons harbouring the vibrio for more than 3 months) have been described, and one individual has been demonstrated to have been infected for 7 years. Chronic carriers are apparently quite rare, however, and their role in the maintenance of cholera infection has not been established.

SPECTRUM OF ILLNESS

The vast majority of individuals infected with *V. cholerae* have no symptoms whatsoever, or only mild diarrhoea. The ratio of severe cases (requiring hospitalization) to mild or inapparent infections has been shown to be about 1: 5 to 1: 10 for classical cholera, and only about 1: 25 to 1:100 for El Tor cholera. With both forms of the disease, therefore, the hospitalized cases represent the tip of an iceberg, and the vast majority of infections will remain undetected unless intensive bacteriological or serological studies are made. Because of the high frequency of inapparent infection and mild disease, isolation and quarantine practices have proved ineffective in the control of cholera epidemics.

THE ROLE OF WATER, FOOD, FOMITES, AND FLIES IN CHOLERA TRANSMISSION

V. cholerae tolerates poorly such environmental stresses as drying, exposure to sunlight, and competition with other organisms. Water is found to be contaminated only in association with infected individuals; contaminated water will become free of vibrios in a few days if such individuals are removed. *V. cholerae* will remain viable for several days in food that is alkaline and moist, provided that competing organisms do not overgrow it. Flies may physically transport vibrios from excreta to food, although they have not been shown to play a significant role in the spread of cholera. Vibrios may remain viable for long periods of time in clothing or bedding contaminated with cholera stool, and

these items have been implicated in the transmission of infection where water in which they are washed was subsequently used for drinking. Fomites are not important in transmission. There is no epidemiological evidence to justify some of the measures often taken against cholera, such as prohibiting the importation of items varying from stamped envelopes to timber.

CHAPTER 4

SURVIVAL OF CHOLERA VIBRIOS IN FOOD, WATER AND FOMITES

D. BARUA[a]

The viability of vibrios outside the human host is of great epidemiological significance, as repeated epidemiological studies have indicated that cholera is generally transmitted from one person to another either directly or through the environment. Since discovery of the causative organism of cholera, many investigations have been carried out on the survival of *V. cholerae* in the environment, but unfortunately there has been no uniformity in the type and state of the contaminating agent, method of sampling, experimental conditions of temperature and pH, techniques of isolation of vibrios from the contaminated samples, etc. It is now known that the survival of vibrios in the environment depends on several factors, such as degree of contamination, temperature, pH, osmotic pressure, moisture content, salt and carbohydrate concentration, and presence of organic matter and of other bacterial flora. Some of these factors have recently been determined with great care by Miyaki et al. (1967).

A great deal of information on this aspect is available in the published works of Shousha (1948), Pollitzer (1959), Felsenfeld (1965), Neogy (1965), Pesigan et al. (1967), Pandit et al. (1967), and Prescott & Bhattacharjee (1969). In spite of the differences in the experimental conditions it is not difficult to see that vibrios have only a limited capacity to survive in the environment. It has also been possible to summarize the relevant information in a table, showing the periods of survival of *V. cholerae* and *V. cholerae* biotype El Tor under various experimental conditions. The longest periods of survival have been given, as *V. cholerae* biotype El Tor is known to be more hardy and resistant to environmental factors.

Many kinds of vegetables and fruits obtained from markets located in the cholera endemic foci have been repeatedly examined for vibrios with no success. They include onions, tomatoes, aubergines (eggplants),

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VIABILITY OF *V. CHOLERAE* AND *V. CHOLERAE* BIOTYPE EL TOR IN FOOD, WATER, AND FOMITES

Articles	Period of survival (days)	
	At room temperature (30–32°C)	At refrigeration temperature (5–10°C)
Cooked food:		
Rice, noodles, fried vegetable rolls, fish, meat, gruel, rice cake, fried sprouts, shrimps, sausages, eggs, cereals, sweet potatoes, tapioca, spinach, tomatoes, peas, potatoes	2–5	3–5
Fresh vegetables:		
Tomatoes, onions, aubergines (eggplants), peas, celery, green beans, bean sprouts, okra, pumpkins, potatoes, cabbage, cucumbers, melons, lettuce, carrots, cauliflower, garlic, peppers, squash, parsley, sweet corn (maize)	1–7	7–10
Fish and shellfish:		
Salted shrimps, shellfish, oysters, fish-meat, smoked fish, dried fish	2–5	7–14[a]
Fruits:		
Atis, guavas, bananas, mangoes, limes, oranges, pomelos, tangarines, mangosteen, melons, papayas, pineapples	1–3	3–5
Dried fruits:		
Dates, figs, raisins, peanuts, walnuts, hazelnuts	1–3	–
Beverages:		
Beer, cola drinks, carbonated water	1	1
Milk and milk products:		
Milk, ice-cream, butter	7–14	14 or more
Grains:		
Rice, pulse, wheat, lentils	1–3	3–5
Spices:		
Red chili, turmeric, cardamom, cinnamon, caraway seeds, peppercorns, ground pepper, bay leaf, ginger root	1–5	
Sweets:		
Milk sweets (sandesh, rossogolla)	1–2	
Others:		
Coffee (ground), tea-leaves, curd (yoghourt)	< 1	
Rice soaked overnight in water	1 hour	
Tank or well water	7–13	18
Sea water	10–13	60
Fomites:		
Aluminium foil, coins, paper, coal, cement, metals, ores, varnished surfaces	1–2	
Cotton, silk, tobacco, rubber, plastics, leather	3–7	

[a] More than 3 weeks in meat and fish kept in a freezer.

peas, celery, bean sprouts, green beans, potatoes, pumpkins, papayas, bananas, guavas, dates, figs, raisins, grapes, lemons, and oranges.

In endemic areas of India it has hardly ever been possible to isolate *V. cholerae* from natural surface water sources, such as rivers and tanks, in the absence of cases of cholera in the neighbourhood.

It is clear from the many investigations that have been carried out that the survival time of vibrios in water depends on a number of factors such as the temperature and pH of the water and its bacterial, salt, and organic content. Vibrios do not survive long in surface water, particularly in warm weather, unless it is repeatedly contaminated. In clean water taken from a tank or a well and stored in the laboratory, vibrios generally do not survive for more than 7–13 days. In water containing a large number of other bacteria (for example, river water), the survival time of vibrios is very short, about 1 or 2 days, but in sterile, filtered or autoclaved water the survival time varies from 17 days at room temperature to 42 days at 5–10°C

It is interesting to note that the viability of *V. cholerae* El Tor was much shorter in material contaminated with stools (containing $10–10^3$ organisms per gram) from asymptomatic vibrio-excretors than in material contaminated with stools from cholera cases (Pesigan et al., 1967). Excreta from carriers are more likely to contaminate the environment than the stools of cholera patients, who are generally too incapacitated to disseminate infection.

These observations indicate that environmental contamination can play a role in the spread of cholera, though only for a limited period of time and at fairly close range, unless there is repeated contamination by a human source.

REFERENCES

Felsenfeld, O. (1965) *Bull. Wld Hlth Org.*, **33**, 725–734
Miyaki, K. et al. (1967) *Bull. Wld Hlth Org.*, **37**, 773–778
Neogy, K. N. (1965) *Bull. Calcutta Sch. trop. Med.*, **13**, 10–11
Pandit, C. G. et al. (1967) *Bull. Wld Hlth Org.*, **37**, 681–685
Pesigan, T. P., Plantilla, J. & Rolda, M. (1967) *Bull. Wld Hlth Org.*, **37**, 779–786
Pollitzer, R. (1959) *Cholera*, Geneva (*World Health Organization: Monograph Series*, No. 43)
Prescott, L. M. & Bhattacharjee, N. K. (1969) *Bull. Wld Hlth Org.*, **40**, 980–982
Shousha, A. T. (1948) *Bull. Wld Hlth Org.*, **1**, 343–422

CHAPTER 5

CLASSIFICATION AND CHARACTERISTICS OF VIBRIOS

RIICHI SAKAZAKI[a]

In the past, a wide variety of Gram-negative, polar-flagellated rods have been classified as belonging to the genus *Vibrio*. Thirty-four species are described in Bergey's *Manual of Determinative Bacteriology*, 7th edition (1957), and 207 species, of which 169 are legitimate, are listed in *Index Bergeyana* (1966), because their long axis indicated some curvature. Recently, however, some criteria have been established for the taxonomy of the genus *Vibrio* (Davis & Park, 1962; Sebald & Véron, 1963; Sakazaki, Iwanami & Fukumi, 1963), and the International Sub-Committee on Taxonomy of Vibrios has recommended a provisional definition (Feeley, 1966) according to which the majority of species previously classified as vibrios are excluded from the genus *Vibrio*. On the other hand, taxonomic studies on the related organisms have indicated a close relationship between the three genera *Vibrio*, *Aeromonas*, and *Plesiomonas* (Ewing, Hugh & Johnson, 1961; Habs & Schubert, 1962). In the old taxonomy, the genus *Vibrio* was classified as belonging to the family Spirillaceae. Eddy & Carpenter (1964) and Véron (1965) suggested that the genus *Vibrio* might be placed in a new family, *Vibrionaceae*, which should also include the genera *Aeromonas* and *Plesiomonas*. It is important to consider these allied organisms when classifying and identifying vibrios.

DEFINITION OF THE GENUS *VIBRIO*

As mentioned above, the International Sub-Committee on Taxonomy of Vibrios recommended a provisional definition for the genus *Vibrio*. However, this description was unsatisfactory in that it did not permit any distinction between anaerogenic aeromonads, plesiomonads, and vibrios. For this reason, Sakazaki, Gomez & Sebald (1967) suggested a revised description of the genus *Vibrio* based on the distinguishing features summarized in Table 1.

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TABLE 1. CHARACTERISTICS OF *VIBRIO* AND ALLIED GENERA*

Characteristic	*Vibrio*	*Aeromonas*	*Plesiomonas*
Gelatin	+	+	−
Voges-Proskauer test	d	d	−
Indol	+	+	−
Lysine decarboxylase	+	−	+
Arginine dihydrolase	−	+	+
Ornithine decarboxylase	+	−	+
Gas from glucose	−	d	−
Mannitol	+	+	−
Inositol	−	−	+
G + C%	42–47	56–62	51–52

* KEY: + = positive, − = negative, d = different reaction.

All are oxidase-positive, polar-flagellated rods.

On the basis of biochemical characteristics, it is possible to differentiate members of the genus *Vibrio* from those of allied genera by four tests (Table 2):

TABLE 2. IDENTIFICATION OF VIBRIOS AND ALLIED ORGANISMS*

Organisms	TSI butt			Oxidase	Lysine	Mannitol
	Acid	Gas	H_2S			
Vibrio	+	−	−	+	+	+
Aeromonas	+	d	−	+	−	+
Plesiomonas	+	−	−	+	+	−
Enterobacteriaceae	+	d	d	−	d	d

* KEY: + = positive, − = negative, d = different reaction.

SUBDIVISIONS OF THE GENUS *VIBRIO*

As mentioned above, recent bacterial taxonomy includes only a few species within the genus *Vibrio*, although in the early days of bacteriology a wide variety of species were so classified. Of those species, *V. cholerae* and *V. parahaemolyticus* are important in medical bacteriology as vibrios that infect human beings. On the other hand, many unnamed vibrios isolated from sea-water and sea-fish may sometimes be confused with vibrios that infect humans.

Vibrio cholerae

Hugh (1965) suggested that *Vibrio eltor* should be put under *Vibrio cholerae* as *V. cholerae* biotype *eltor*. His suggestion was supported by the International Sub-Committee on Taxonomy of Vibrios (Feeley, 1966). Although the El Tor biotype of cholera vibrio is characterized by its haemolytic activity, some workers use the name for nonhaemolytic strains that are resistant to Mukerjee group IV phage and/or polymixin B, and/or are positive in the chicken red cell agglutination test, since the haemolysis test does not always give reproducible results and, furthermore, many strains of the vibrios from recent epidemics have been nonhaemolytic when tested by the usual methods.

Many methods have been devised for carrying out haemolysis tests of the El Tor biotype. Feeley & Pittman (1963) recommended a method using deep culture of heart infusion broth. Barua & Mukerjee (1964) reported that glycerolized brain-heart infusion was best. However, Sakazaki & Tamura (unpublished data, 1970) confirmed that reproducible results were not obtained in any broth culture, including heart infusion broth and glycerolized brain-heart infusion. They found that the anaerobic plate method, using heart infusion agar, and the aerobic plate method, using brain-heart infusion agar containing 0.05% of sodium thioglycollate and 0.025% (—)-cystine to which washed sheep red cell suspension is added, are the best for obtaining reproducible results.

Feeley (1965) subdivided the cholera vibrio into several biotypes by using tests of sensitivity to Mukerjee group IV phage, plate and tube haemolysis, and chicken red cell agglutination, and emphasized the epidemiological significance of these biotypes. However, the tube haemolysis test is a problem, as mentioned above. Sen (1969) has also subdivided cholera vibrios, using five tests: sensitivity to Mukerjee group IV phage and to polymixin B, tube haemolysis, CCA, and Voges-Proskauer reaction. However, the results of the latter test are often not reliable. Heiberg (1936) described six groups based on the fermentation patterns in mannose, sucrose, and arabinose; Smith & Goodner (1965)

added two further groups. However, it has been emphasized by Hugh (1965) and by Sakazaki, Gomez & Sebald (1967) that the Heiberg tests are not very helpful for identification of the cholera vibrio or of other vibrios that infect humans.

Vibrio parahaemolyticus

Vibrio parahaemolyticus is the name proposed by Sakazaki, Iwanami & Fukumi (1963) for an enteropathogenic, facultatively halophilic organism encountered in Japan as a causative agent of food poisoning. Recently, it has been found that the vibrio is also isolated frequently from diarrhoeal cases in India (Sakazaki et al., 1970). It grows in the presence of 7–8% NaCl in peptone water and does not agglutinate with cholera 0–1 sera.

Sakazaki (1968) described another halophilic vibrio, named *V. alginolyticus*, which, unlike *V. parahaemolyticus*, ferments sucrose. The vibrio is found mainly in sea-water and sea-fish, but sometimes also in human faeces. Its pathogenicity for man is not known.

So-called "NAG" vibrios

There has been some controversy over the terminology of the "NCV" (non-cholera vibrio) or "NAG" (non-agglutinable) vibrio. These vibrios possess biochemical and morphological characteristics very similar to those of the cholera vibrio but are non-agglutinable with polyvalent O serum of the cholera vibrio. Such vibrios are agglutinable by their own antisera and may produce cholera-like or mild diarrhoea. These two terms are both unsatisfactory but they are used for the sake of convenience.

Gardner & Venkatraman (1935) studied the "cholera group of vibrios" which consisted of biochemically similar vibrios possessing the same H antigens as the cholera vibrio. In their study, cholera vibrio belonged to O-subgroup 1, and vibrios not agglutinating with O-subgroup 1 antiserum belonged to other O-subgroups; these were later to be called NAG vibrios. Sakazaki et al. (1970) also demonstrated that the flagellar antigen of all strains of NAG vibrios originating from human sources was identical to that of the cholera vibrio.

Certain difficulties arise from the fact that (*a*) no definition has been given of the R-form of cholera vibrio and (*b*) the mucoid strain of cholera vibrio is also non-agglutinable with homologous O antiserum in the living state. Mucoid strains of cholera vibrio may be wrongly identified as NAG vibrios.

It is therefore suggested that the vernacular name "NAG vibrio" or "NCV" should be used only for those organisms that (*a*) conform to the definition of the genus *Vibrio* according to Table 2, (*b*) possess H antigen

identical to that of the cholera vibrio, and (*c*) are non-agglutinable with O and R antisera of the cholera vibrio even when a culture boiled at 100°C for two hours is used.

REFERENCES

Barua, D. & Mukerjee, A. C. (1964) *Bull. Calcutta Sch. trop. Med.*, **12**, 147

Breed, R. S., Murray, E. G. D. & Smith, N. R. (1957) *Bergey's Manual of Determinative Bacteriology*, 7th ed., Baltimore, Md., Williams & Wilkins

Buchanan, R. E., Holt, J. G. & Lessel, E. F. (1966) *Index Bergeyana*, Baltimore, Md., Williams & Wilkins

Davis, G. H. G. & Park, R. W. A. (1962) *J. gen. Microbiol.*, **27**, 101

Eddy, B. P. & Carpenter, K. P. (1964) *J. appl. Bact.*, **27**, 96

Ewing, W. H., Hugh, R. & Johnson, J. G. (1961) *Studies on the* Aeromonas *group*, Communicable Disease Centre, US Department of Health, Education and Welfare, Atlanta, Ga., USA

Feeley, J. C. (1965) *J. Bact.*, **89**, 665

Feeley, J. C. (1966) *J. system. Bact.*, **16**, 135

Feeley, J. C. & Pittman, M. (1963) *Bull. Wld Hlth Org.*, **28**, 347

Gardner, A. D. & Venkatraman, K. V. (1935) *J. Hyg. (Lond.)*, **35**, 262

Habs, H. & Schubert, R. H. W. (1962) *Zbl. Bakt., I. Abt. Orig.*, **186**, 316

Heiberg, B. (1936) *J. Hyg. (Lond.)*, **36**, 114

Hugh, R. (1965) *Int. Bull. bact. Nomencl.*, **15**, 61

Sakazaki, R. (1968) *Jap. J. med. Sci. Biol.*, **21**, 359

Sakazaki, R., Gomez, C. Z. & Sebald, M. (1967) *Jap. J. med. Sci. Biol.*, **20**, 265

Sakazaki, R., Iwanami, S. & Fukumi, H. (1963) *Jap. J. med. Sci. Biol.*, **16**, 161

Sakazaki, R., et al. (1970) *Jap. J. med. Sci. Biol.* (in press)

Sebald, M. & Véron, M. (1963) *Ann. Inst. Pasteur*, **105**, 897

Sen, R. (1969) *Indian J. med. Res.*, **57**, 856

Smith, H. L. & Goodner, K. (1965) In: *Proceedings of the Cholera Research Symposium, Honolulu, 1965*, Washington, D.C., US Government Printing Office (PHS Publication No. 1328)

Véron, M. (1965) *C. R. Acad. Sci. (Paris)*, **261**, 5243

CHAPTER 6

CHOLERA PHAGES

S. MUKERJEE[a]

INTRODUCTION

The presence of bacteriophage in the stools of cholera patients was first observed by d'Herelle in 1920, and confirmed shortly afterwards by several other authors. The historical development of studies on cholera bacteriophage has been reviewed by Pollitzer (1959) in his monograph on cholera. Since 1927, when d'Herelle initiated work on the cholera bacteriophage, intensive studies have been carried out by a number of renowned scientists in India. During the course of these investigations, 14 types of cholera bacteriophage (A–N) were discovered and studied. Most of these bacteriophages, including the most important, the type A phage of Aseshov, are no longer available. Those that are still available show evidence of variation. The primary aim of many of these studies was to investigate the possibility of using bacteriophages in the prophylaxis and treatment of cholera. Both favourable and unfavourable results were reported. Ultimately, the use of cholera bacteriophage for these purposes was abandoned, and there was a general pause in phage research.

With the return of interest in phage, new aspects of cholera bacteriophage research have received attention, as a result of which bacteriophage has emerged with a very important position in cholera studies. In this paper, the uses of cholera bacteriophage as a tool for laboratory diagnosis and a guide for epidemiological studies are examined. The present status of bacteriophage in the treatment and prophylaxis of cholera is also discussed.

BACTERIOPHAGE-TYPING OF CHOLERA VIBRIOS

Bacteriophages are known to possess different spectra of lysis for different strains belonging to the same species of bacterial host. By

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studying their comparative sensitivity to bacteriophages, it is thus possible to distinguish between strains of bacteria that are otherwise indistinguishable by laboratory techniques, including antigenic analysis. It has also been found that strains of bacteria isolated from different outbreaks originating from a common source behave in an identical manner in the presence of typing phages. This has led to utilization of the technique of phage-typing for identification of bacterial strains in epidemiological studies to determine the source and routes of spread of an infectious disease. Phage-typing techniques have been developed for a number of bacteria and phage-typing is now considered an indispensable aid in the control of enteric infections.

Cholera phage-typing has aroused international scientific interest. In 1964, an International Reference Centre for Vibrio Phage-typing [1] was established in Calcutta under the auspices of the World Health Organization. Cholera phage-typing techniques have been developed in recent years and may be considered under the following headings:

1. *Phage-typing of* V. cholerae

The classical cholera vibrio strains have so far been classified into five principal types with the help of four groups of cholera bacteriophages (Mukerjee, 1963). The susceptibility patterns of these types are given in Table 1.

TABLE 1. THE PATTERNS OF SENSITIVITY OF FIVE PHAGE-TYPES OF *V. CHOLERAE*

Phage-types of *V. cholerae*	Sensitivity to phage group			
	I	II	III	IV
1	+	+	+	+
2	−	+	+	+
3	+	−	+	+
4	−	−	+	+
5	+	+	−	+

+ = sensitive
− = insensitive

[1] Recently renamed "International Reference Centre for Vibrios".

In addition, three sub-types have been identified in each of the types 1 and 2. No correlation has been found to exist between phage-types and serotypes of *V. cholerae.*

The phage and serological types of causative vibrios have been found to vary considerably in Calcutta in the course of one year. This is possible because of the absence of chronic carriers of cholera vibrios.

The number of types and sub-types of *V. cholerae* is comparatively small, and some of the recognized types cannot be isolated from cases at present. However, the practical value of this scheme of phage-typing in epidemiological studies of cholera has been established. It has been found that there are at least three different endemic cholera foci in India harbouring different phage types and serotypes of *V. cholerae.* For proper control and final eradication of cholera in this country, it would be essential to organize control measures simultaneously in all these areas.

2. *Phage-typing as a means of differentiating* V. cholerae *and El Tor vibrio strains*

V. cholerae and El Tor vibrios closely resemble each other in morphology, biochemical reactions, and antigenic structure. They are usually differentiated by the haemolysis test, *V. cholerae* being non-haemolytic to sheep erythrocytes while El Tor vibrios are haemolytic. Since the beginning of the present El Tor pandemic, difficulties have frequently been experienced by laboratory workers due to the emergence of non-haemolytic El Tor vibrios. As a matter of fact, almost all the El Tor strains isolated in recent outbreaks are devoid of haemolytic property. This inconsistency of haemolytic character has emphasized the need for a dependable test for identification of El Tor vibrios.

The possibility of utilizing the differences in phage-susceptibility patterns of *V. cholerae* and El Tor vibrio strains for differentiation purposes was first reported in 1960 (Mukerjee, 1960a, b; Newman, 1960). A test was developed on the basis of the observation that a group IV cholera phage at its routine test dilution was consistently lytic for all strains of *V. cholerae* and for none of the El Tor vibrio strains. In extended studies, the phage-susceptibility test has yielded the most consistent results in differentiating *V. cholerae* and El Tor vibrios (Mukerjee, 1963). Further observations showed that all El Tor strains which appeared to be non-haemolytic but were identified on the basis of phage IV resistance proved to be frankly haemolytic when tested after glycerol or an inhibitor of proteolytic enzyme had been added to the heart-infusion-broth medium. However, the patterns of reactions to tests for phage-typing, polymyxin sensitivity, and haemagglutination should give an ultimate identification of any atypical strain. In order to obtain

the best results, the group IV phage must be maintained properly and tested at its routine test dilution for *V. cholerae.*

3. *Phage-typing of El Tor vibrios*

The pandemic spread of cholera El Tor in the Western Pacific Region and South, South-East, and Central Asia since 1961 has stimulated interest in methods of identifying strains of El Tor vibrio for epidemiological purposes. Phage-typing of El Tor vibrios is now more important than phage-typing of *V. cholerae.* However, owing to the high mutation rates in El Tor strains, it has not been possible to finalize the tentative scheme for El Tor phage-typing developed recently (Mukerjee, 1965), in spite of persistent attempts. Purification of the typing phages by repeated pure-line isolations has enabled a more stable typing scheme for the El Tor vibrios to be developed. This scheme uses five typing phages active against El Tor vibrios. The lysis patterns of El Tor vibrio strains are given in Table 2. Some of these strains showed divergent phage-sensitivity patterns when tested repeatedly with the same sets of phages. However, this difficulty is being overcome by adding to or altering some of the typing phages in the tentative scheme.

TABLE 2. THE PATTERNS OF SENSITIVITY OF SIX PHAGE-TYPES OF EL TOR VIBRIO

Phage-type of El Tor vibrio	Sensitivity to phage group				
	I	II	III	IV	V
1	+	+	+	+	+
2	+	+	+	−	+
3	+	+	−	+	+
4	+	+	−	−	+
5	+	−	−	−	+
6	−	+	−	−	+

4. *Pro-phage-typing of cholera vibrios*

The occurrence of lysogenicity in cholera vibrios has been known for a long time. The vast majority of *V. cholerae* strains of recent origin have been found to be non-lysogenic, while all but a few El Tor strains are

lysogenic. It has not been possible to develop a phage-typing scheme based on lysogenicity patterns of El Tor strains. Takeya & Shimodori (1963) believe that there might be a causal relationship between lysogenicity and virulence of El Tor vibrios, but this possibility has not been established. On the other hand, El Tor strains isolated from the stools of cholera patients have often been found to be non-lysogenic; all the El Tor cultures from recent outbreaks in Thailand and Cambodia were non-lysogenic. In other areas, also, non-lysogenic El Tor strains are not uncommon. In India, for example, 25% of all El Tor cultures isolated in the past two to three years were non-lysogenic. In fact, lysogenicity is found to be absent in all strains belonging to phage-type 1.

THERAPEUTIC AND PROPHYLACTIC USES OF CHOLERA BACTERIOPHAGES

The causative organisms of cholera are confined to the gut of the patient. Theoretically speaking, therefore, cholera would appear to be a very suitable disease for the application of phage therapy. Bacteriophage therapy of cholera, if successful, would be of immense value, particularly in areas where facilities for prompt treatment with saline are still lacking. In a series of studies that began in 1928 and extended over nearly two decades, favourable as well as unfavourable results were obtained in the use of cholera bacteriophage for treatment and prophylaxis of cholera. However, recent trials in East Pakistan have demonstrated conclusively that cholera phages are of practically no therapeutic value.

Annex

PRACTICAL METHODS FOR BACTERIOPHAGE WORK

1. Phage isolation

(*a*) *From stools*

To 50 ml of nutrient broth medium in a flask add 1–2 ml of liquid stool and 1 ml of visibly turbid young broth culture of a propagating strain of vibrio. Incubate it at 37°C overnight. Filter through a candle or any other bacterial filter. Bacteriophage, if present, will be found in the filtrate.

(*b*) *From water*

To 190 ml of water sample add 10 ml of 20% peptone water. Correct the reaction of the mixture to a slightly alkaline pH. Add 1 ml of a 2–4-hour broth culture of the indicator strain. Incubate and filter as in (*a*).

(c) *From lysogenic bacteria*

In some cases, bacteriophage may be isolated from a broth culture of lysogenic bacteria; in other cases, it is obtained only when it is grown with a sensitive strain. When phage is liberated by a lysogenic bacterium, it produces lysis of the sensitive strain. The host strain liberating the phage remains insensitive to it. The lysogenicity of a strain can usually be detected by spotting it on the lawn of a sensitive culture.

2. Agar layer method of plating a phage

Prepare 10-ml quantities of 0.7% agar medium in test tubes. At the time of plating a phage, immerse the tubes containing the soft agar in a boiling water bath and cool to 46 °C. Add 0.1 ml of the phage filtrate at the desired dilution, followed by one or two drops of the visibly turbid young broth culture of bacteria, to each of the tubes. Pour the content of each tube on to an agar plate. Mixing and even distribution of the phage and bacteria are facilitated by rocking the plate. Leave the plates on the table to allow the soft agar to set. Incubate the plates overnight. The phage plaques will appear as transparent areas in the opaque layer of bacterial growth.

3. Identification of phage

This may be done by noting the lysis patterns of the phage for the phage-types of vibrio strains and phage-resistant cultures.

4. Routine test dilution of a phage

Ten-fold dilutions of the phage preparation in broth are spotted on the spot or streak culture of a sensitive strain of vibrio. After overnight incubation, note the highest dilution which causes confluent lysis. This dilution is known as the critical test dilution or routine test dilution (RTD) of the phage.

5. Routine procedure of phage-typing

The technique is designed to obtain areas of uniform inoculation of strains of bacteria under test, on which standard volumes of the phage filtrates at their routine test dilutions are superimposed.

With the help of calibrated platinum loops (3 mm in diameter), make circular spotted cultures of about 1.2 cm diameter on previously marked areas on agar plates. Add standard volumes of typing phages to the cultures. After overnight incubation, take the reading. The different degrees of reaction are recorded as follows:

Clear or confluent lysis	Cl
Less than confluent lysis with a few discrete bacterial colonies	Cl or Lcl
Semi-confluent lysis	Scl
Different degrees of discrete plaque formation	plq. +++, ++, +
Opaque lysis with evidence of growth inhibition	Ol
No lysis	—

REFERENCES

Mukerjee, S. (1960a) *Ann. Biochem.*, **20**, 317
Mukerjee, S. (1960b) *J. Hyg. (Lond.)*, **59**, 109
Mukerjee, S. (1963) *Bull. Wld Hlth Org.*, **28**, 337
Mukerjee, S. (1965) In: *Proceedings of the Cholera Research Symposium, Honolulu, 1965*, Washington, D.C., US Government Printing Office (PHS Publication No. 1328)
Newman, F. S. (1960) *Bact. Proc.*, p. 77
Pollitzer, R. (1959) *Cholera*, Geneva, World Health Organization (*Monograph Series*, No. 43)
Takeya, K. & Shimodori, S. (1963) *J. Bact.*, **85**, 957

CHAPTER 7

LABORATORY DIAGNOSIS OF CHOLERA CASES AND CARRIERS

D. BARUA[a]

It is not necessary, for treatment purposes, to confirm the diagnosis of typical cases of cholera by laboratory examination. Mild cases, however, cannot be diagnosed without such additional investigation. Confirmation of the diagnosis by laboratory methods is also necessary to enable public health administrators to obtain resources for the control of cholera. While it is most important to have confirmation of an outbreak as quickly as possible, past experience has shown that diagnosis of the first cases usually takes an unduly long time. This is mainly due to lack of preparation on the part of the health services of the country.

LABORATORY DIAGNOSIS OF CHOLERA CASES

The presence of a large number of vibrios (10^7–10^9/ml) in the stools of cholera cases renders diagnosis relatively simple and less demanding.

Collection of specimens

Stool samples collected early in the course of the disease, particularly before administration of any antimicrobial drug, are the most rewarding material. The specimen is best collected using a No. 26 or 28 rubber catheter lubricated with sterile paraffin and passed into a sterile screw-capped vial or test tube. A rectal swab or glass rod is often used for the sake of convenience, but it is essential to make sure that the swabbing is done properly. The rectal swab should be made of good quality cotton-wool which will absorb about 0.1–0.2 ml of fluid. Contamination from the perineum should be avoided. Before swabbing convalescents who no longer have watery diarrhoea, it is preferable to moisten

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the swab with peptone water, removing any excess fluid by pressing the swab against the side of the tube. In rural areas, where samples are often taken from the stool collection pan, the organism may be carried over from one sample to another if a clean pan is not used on each occasion; on the other hand, the organism may be killed by the disinfectants generally used in such pans.

Methods for rapid diagnosis

Although a quick method of diagnosis is not needed for treatment purposes, it would greatly facilitate the planning of control measures, including quarantine, and of epidemiological or clinical investigations.

Bandi's procedure of incubating cholera stools for 2–7 hours in peptone water with anti-cholera serum has not been practised since Ghosal & Paul found that routine cultural methods give a 38% higher yield of positive results.

By dark-field microscopy about 80% of the cases can be correctly diagnosed within a few minutes if the examination is done early in the course of the disease; after 6–18 hours' enrichment of faecal matter, even more can be diagnosed. No special skill or equipment is required. A portable dark-field microscope (particularly the Cooke-McArthur model) is a practical instrument for field diagnosis. The fluorescent antibody technique can also diagnose around 90% of the cases correctly in about two hours, but it needs special skill and equipment. Both methods are complementary to the usual method of isolation.

It is possible, however, to obtain a correct bacteriological diagnosis in 4–5 hours with the help of a stereoscope. The faecal matter is properly streaked on a dry, non-inhibitory nutrient agar plate with or without 0.1% of Teepol, and incubated for 4–5 hours. The vibrio colonies can then be easily spotted with a stereoscope, and confirmed by slide agglutination with O-Group serum. This procedure has been tried with total success on more than 300 specimens collected within the first two days of disease. The stereoscope used alone is not dependable.

Transportation of the specimen

Venkatraman-Ramakrishnan's fluid (10 ml) may be used when at least 1–3 ml of the inoculum are available. If the inoculum is small, as in the case of a rectal swab (about 0.1 ml), it should be transported in alkaline trypticase taurocholate tellurite broth (TBB—pH 9.2) or in alkaline peptone water (PW—pH 8.0–8.5), both of which have enriching as well preservative properties. The final concentration of potassium tellurite in TBB should be about 1: 100 000 to 1: 200 000, and each

batch of tellurite has to be standardized. The presence of even a single vibrio can be detected by enrichment in either of these two fluids. The simple method of preparation of alkaline peptone water, its low cost, and its good performance in the field make this the most suitable medium. It should be distributed in screw-capped or rubber-capped bottles, rather than in test tubes. In tropical climates, the vibrios may be overgrown by commensals when kept for a long time before plating; this difficulty, if it arises, can be overcome by transferring about 0.1–0.2 ml to a fresh tube of PW or TBB on arrival in the laboratory, and incubating for 6–8 hours. For a second enrichment, TBB was found to be superior to PW, particularly when incubated for 24 hours. Cholera vibrios were found to remain viable for 2–6 weeks or more in samples collected in PW and kept at room temperature; there was no loss of vibrio within 14 days in any of the 35 samples tested.

Teepol-peptone water (alkaline peptone water with 0.1–0.2% of Teepol) prevents the growth of cocci and aerobic spore-bearers; vibrios remain viable for a longer period, but their growth is slow. This may be a more suitable medium for transportation over a considerable distance from field to laboratory. Cary-Blair medium, which has been found to be a useful transport medium for *Salmonella*, *Shigella*, *Escherichia* and *Yersinia pestis*, is also good for *V. cholerae*.

If no media are available, strips of unsterile thick blotting paper, as used by students, may be soaked in the stools and placed in an envelope made of ordinary plastic wrapping material, which must be properly sealed to prevent the sample drying out before being sent to the laboratory. Vibrios have been found to survive on such strips for about 5 weeks, or for as long as the moisture is preserved.

Enrichment before plating

In the case of a stool sample collected on the first or second day of illness, direct streaking on a solid medium has yielded better results than enrichment followed by plating, although the latter procedure is better, for obvious reasons, for specimens collected during convalescence. Direct plating has an additional advantage; the associated non-agglutinable vibrios, if any, do not have time to overgrow the agglutinable vibrios as they may do in the tube of enrichment medium. However, both direct streaking and inoculation for enrichment should be routinely performed at the same time whenever possible. For primary enrichment, the simple alkaline peptone water (pH 8.0–8.5) incubated for 8 hours has been found to give better results.

Solid media for plating and isolation

The choice of plating medium depends upon the experience of the individual worker and the facilities available. Some of the well-tried

media are (1) non-inhibitory or slightly inhibitory (nutrient agar, pH 7.6; gelatin agar, pH 8.0; Teepol agar with 0.1% Teepol, pH 7.6, and bile salt agar, pH 8.2), (2) highly selective (TCBS medium, Eiken, pH 8.6; Vibrio agar, Nissui, pH 8.5; and gelatin trypticase taurocholate tellurite agar, pH 8.5). To avoid unpredictable failures it is better, if possible, to use one of each kind of medium for each specimen, one-half of each being used for direct streaking and the other half after enrichment. The selective medium is not essential for cases in their first or second day of illness when it may be more economical to use one or two plates of freshly prepared non-inhibitory medium (not more than 2–3 days old).

The colonies can be spotted with the naked eye after overnight incubation, but they can be picked up with great ease and confidence from transparent medium with the help of a stereoscope using transmitted oblique light. Stereoscopic examination can differentiate vibrio colonies from those of many other species including *Aeromonas*, but not always from the colonies of certain strains of *Pseudomonas*, *Plesiomonas* and non-agglutinable vibrios. It is also time-consuming when dealing with many specimens.

Identification and characterization

Suspected colonies should be tested first with the appropriate dilution of standard anti-cholera O-Group 1 serum and then, if positive, with the Ogawa and Inaba type-specific antisera by slide agglutination. If the suspect colony fails to react with the group serum, at least 5–10 colonies and a sweep from the confluent area should be similarly tested before deciding that the case is negative for agglutinable vibrios, as they may co-exist with the non-agglutinable variety. It may be better to pick up 5–10 such doubtful colonies on Kligler iron agar (KIA) or nutrient agar slants for further study.

The remains of the agglutinable colony should be picked up with a straight wire on a KIA slant which, after overnight incubation, should show the typical biochemical character (acid butt). The growth should be confirmed serologically and then subjected to tests for fermentation of mannose, sucrose and arabinose, production of acetylmethylcarbinol by Barritt's technique, haemagglutination of chicken or sheep cells, and resistance to 50-unit polymyxin B discs and cholera-phage group IV at routine test dilution. When facilities are available, some representative strains from each outbreak should be tested for sensitivity to chloramphenicol and tetracycline using 1.6, 3.2, 6.3 μg/ml, and to streptomycin using a 5 μg/ml concentration by agar plate diffusion technique. A few strains were found to be resistant to these antibiotics in the

Philippines in 1965. Representative strains should be sent to the WHO International Reference Centre for Vibrios in Calcutta.

Serological tests, running paired sera (the acute phase serum being collected within 48 hours of onset and the convalescent serum between the seventh and tenth days), have been found to be very reliable for retrospective diagnosis of cholera. The agglutination test, using live antigen, and the vibriocidal antibody test have been tried on a wide scale and have given results that correspond closely to the bacteriological findings. The serological test may serve as a check on the bacteriological procedures of a laboratory.

LABORATORY DIAGNOSIS OF CHOLERA CARRIERS

Contact and convalescent carriers have been found to excrete intermittently about 10^2–10^5 vibrios per gram of stools, together with a large number of commensals, whereas a cholera patient excretes about 10^7–10^9 vibrios per millilitre, with a few commensals. This makes the diagnosis of a carrier more difficult.

Contacts should be examined as soon as possible after detection of the index case. They should also be followed up daily, as more vibrio excretors are detected among them in this way.

It is generally advisable to inoculate about 3 g of stools into 50–100 ml of alkaline peptone water soon after evacuation, but moistened rectal swabs, glass rods or stool swabs are used for convenience. As vibrios have been found to be more or less evenly distributed on the surface as well as inside the faecal mass, a *properly* collected rectal or stool swab may be reliable. The specimen should be collected in alkaline peptone water with or without Teepol, or in alkaline bile salt tellurite broth in the field. On arrival in the laboratory, it should be incubated for 8 hours and then plated on both selective and non-selective media; about 0.1–0.2 ml should be transferred for second enrichment to PW or, preferably, TBB for proper incubation (24 hours for the latter). Plating should be done from the second tube if it has not been possible to isolate the vibrio from the first. This procedure has yielded about 11% more positive isolates in a group of carriers. It may be difficult to detect small numbers of vibrio colonies on non-inhibitory media without the aid of a stereoscope; the selective media are therefore preferable. The organism should then be identified and characterized in the same way as for patients.

Contact carriers also develop a high antibody titre, but serological examination of paired sera is not a practicable procedure for diagnosis of such carriers.

For the detection of post-cholera carriers, prompt bacteriological examinations of the stools after purging (magnesium sulfate, 15–30 g; or mannitol, 30 g) and of bile after duodenal intubation are of special value. In the absence of vaccination, serological follow-up of former cases may be an easier way to trace post-cholera carriers, but this method is not applicable in highly endemic foci. Persistence of a high antibody titre for 12 weeks or more after the illness and subsequent demonstration of vibrios in the stool and, if possible, in the bile will confirm diagnosis of the carrier state.

CHAPTER 8

PATHOGENESIS AND PATHOPHYSIOLOGY OF CHOLERA

C. C. J. CARPENTER, jr[a]

PATHOGENESIS

The pathogenesis of cholera is basically simple. The cholera victim ingests viable *V. cholerae*. The organisms multiply in the small bowel and produce an exotoxin, which acts upon the mucosal cells of the small bowel, causing them to secrete large quantities of isotonic fluid. The small bowel produces isotonic fluid faster than the colon can absorb it, and the result is a watery isotonic diarrhoea. The rapid gastro-intestinal loss of isotonic fluid is responsible for all the clinical manifestations of the disease.

The critical events in the pathogenesis of cholera are therefore: (1) the delivery of viable organisms to the small bowel, (2) the multiplication of the organisms in the small bowel, (3) the production by the multiplying organisms of a potent exotoxin, and (4) the secretion of isotonic fluid by the mucosal cells of the intestine in response to the toxin.

1. Before the organisms can enter the small gut they must pass through the stomach. Since they are acid-sensitive, it is almost impossible to produce cholera in volunteers (or in the well-known rabbit and canine models of cholera) without first neutralizing the gastric acid. In the normal human volunteer, without prior neutralization of the gastric contents, mild diarrhoea is only occasionally produced by the oral administration of 10^{11} viable pathogenic organisms. After neutralization of gastric contents with sodium bicarbonate, however, moderately severe diarrhoea can be produced fairly predictably in about 50% of volunteers by the oral administration of 1 million viable organisms. This suggests that gastric acid provides the first line of host defence against cholera, and that, in spontaneously occurring cholera cases, there must be at least transient neutralization of gastric acid

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(e.g., caused by a high-protein meal) at the time the vibrios are ingested, in order to allow colonization of the small intestine to take place. No data are yet available on the base-line levels of gastric acidity in persons who develop cholera.

2. *V. cholerae* thrives in isotonic fluid with a pH in the range 7.0–8.0, and therefore generally multiplies rapidly in the small bowel of man and of various experimental animals. However, not all the factors in the small bowel that are helpful or harmful to vibrio growth are known. Even when virulent organisms survive passage through the stomach, they do not invariably thrive in the upper intestine. In his investigations into canine cholera, for example, Sack found that, in animals challenged with *V. cholerae* Ogawa 395, the attack rate of clinical cholera (40–50%) was no greater when the organisms were delivered directly into the duodenum than when they were administered by orogastric tube after neutralization of the gastric contents.

3. The production of an adequate amount of exotoxin to cause electrolyte secretion in the small intestine appears to be dependent largely upon the strain of *V. cholerae* that is multiplying. In experimental canine cholera, attack rates following orogastric challenge with clinical isolates of *V. cholerae* vary from zero to 80%. The attack rate following challenge with a given strain of *V. cholerae* is closely correlated with the ability of that strain to produce exotoxin in a number of culture media *in vitro*. If *V. cholerae* Inaba 569B, which is a very potent producer of exotoxin *in vitro*, colonizes and multiplies in the canine small bowel, severe clinical disease usually ensues. On the other hand, if the canine small bowel is colonized by *V. cholerae* Ogawa 412, which is a very poor exotoxin producer *in vitro*, severe clinical disease rarely, if ever, follows. Once *V. cholerae* has successfully colonized and begun to multiply in the mammalian small bowel, the severity of the resulting disease appears to be dependent upon the ability of the infecting strain to produce exotoxin.

4. The secretion of isotonic fluid by small bowel mucosal cells in response to exotoxin seems the most predictable of the four pathogenetic events leading to clinical cholera. Available data from animal models indicate that these mucosal cells, when exposed to an adequate exotoxin stimulus, respond by a prolonged secretion of isotonic fluid. Although the dose of exotoxin required for a maximum secretory response varies greatly from species to species, the dose is fairly constant for animals in the same species. Following a single, brief (less than 10 minutes) exposure to the exotoxin, the small bowel of the experimental animal secretes isotonic fluid for a period of 12–24 hours. This secretion is apparently triggered by an interaction between the exotoxin and some enzyme system in the gut epithelial cells, but the mechanism is not yet

known for certain. Available data indicate that the exotoxin has no direct effect on any organ beyond the epithelial cells. Specifically, there is no histological evidence that the toxin affects capillaries of the intestinal lamina propria, and the electrolyte secretion does not appear to be related to any alterations in arterial blood flow to the gut. Moreover, it has been impossible, even by the most sensitive bio-assay techniques, to demonstrate any exotoxin in either thoracic duct lymph or superior mesenteric venous effluent during experimentally induced cholera.

An area of considerable importance, about which little is known, is the effect of underlying disease states on the response of the small bowel to exotoxin. In sprue, for example, when the absorptive function of the small bowel is impaired, it is not known whether the effect of the exotoxin is heightened because if inadequate electrolyte absorption in the face of exotoxin-induced electrolyte secretion, or whether the effect is diminished because of a poor secretory response of the abnormal gut epithelial cells.

Thus, although the basic steps in the pathogenesis of cholera are clearly delineated, certain factors that are important to each of these steps remain obscure. It seems potentially very important to determine which characteristics of the host's small intestine are essential for, and which are antagonistic to, the multiplication of *V. cholerae.*

PATHOPHYSIOLOGY

The pathophysiological changes in the cholera patient result directly from the massive gastro-intestinal loss of an isotonic fluid with a low protein content (less than 200 mg per 100 ml) and with the following electrolyte concentrations (mean values $\pm$ standard deviation, mEq/litre): sodium 126 $\pm$ 9, potassium 19 $\pm$ 9, bicarbonate 47 $\pm$ 10, and chloride 95 $\pm$ 9. The loss of this fluid, sometimes at the rate of one litre per hour in the adult, rapidly leads to hypovolaemic shock and metabolic acidosis with the typical associated physical and laboratory abnormalities. The physical findings include apathy, cyanosis, thready or absent peripheral pulses, very poor skin turgor with scaphoid abdomen, sunken eyes and "washerwoman's hands", and weak or inaudible heart sounds. The laboratory abnormalities include severe metabolic acidosis, haemoconcentration, and marked elevation of the plasma protein concentration. If the cholera patient is treated properly, with prompt intravenous infusion of fluid and electrolytes in quantities equal to the gastro-intestinal losses, all the physical and biochemical abnormalities are rapidly reversed. Delayed or inadequate treatment may result in two

additional pathophysiological alterations: acute renal failure and the problems associated with hypokalaemia.

Acute renal failure, with the pathological features of acute tubular necrosis, may occur if treatment is initiated only after the patient has been in hypovolaemic shock for a considerable period of time, or if hypovolaemic shock recurs as the result of inadequate intravenous fluid therapy after initial rehydration. This clinical syndrome is usually reversible with judicious fluid management, but presents two major differences from acute tubular necrosis in the absence of cholera, viz., a more severe degree of metabolic acidosis and a less severe degree of hyperkalaemia. The metabolic acidosis is more severe because the cholera patient is already acidotic as the result of gastro-intestinal bicarbonate loss at the time renal failure develops, and it becomes progressively more severe as the renal failure persists. Hyperkalaemia is a less severe problem in that the cholera patient is potassium-depleted, again as the result of gastro-intestinal losses, at the time renal failure develops, and thus the failure of renal potassium excretion initially serves only to restore plasma potassium levels to normal and only occasionally results in serious degrees of hyperkalaemia.

Serious hypokalaemia is a greater problem in paediatric patients than in adults, for young children appear to be more susceptible to the cardiac and gastro-intestinal consequences of potassium loss. Clinically significant potassium deficiency usually results from continued losses of potassium in stool, in cases where replacement fluids contain insufficient potassium. If active purging continues for several days, and no potassium replacement is given by either the oral or the intravenous route, deficits of as much as one-third of total body potassium may occur, leading to atonic bowel, cardiac arrhythmias, hypotension, and ultimately to cardiac arrest. In such instances, pathologically distinctive lesions are seen in both the myocardium and the kidneys. Such severe potassium deficiency should not occur, however, as potassium can be replaced by either the oral or the intravenous route; the potassium requirements of the actively purging cholera patient have been clearly determined by a number of investigations.

RELEVANT LITERATURE

Carpenter, C. C. J., Chaudhuri, R. N. & Mondal, A. (1964) *Indian J. med. Res.*, **52**, 924

Chaudhuri, R. N. & Carpenter, C. C. J. (1968) *J. Indian med. Ass.*, **51**, 182

De, S. N. & Chatterjee, D. N. (1953) *J. Path. Bact.*, **66**, 559

De, S. N., Ghose, M. L. & Sen, A. (1960) *J. Path. Bact.*, **79**, 373

Greenough, W. B. & Carpenter, C. C. J. (1969) *Tex. Rep. Biol. Med.*, **27**, 203

Phillips, R. A. (1963) *Bull. Wld Hlth Org.*, **28**, 297

Sack, R. B. & Carpenter, C. C. J. (1969) *J. infect. Dis.*, **119**, 150

CHAPTER 9

THE CLINICAL PICTURE OF CHOLERA

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The spectrum of diarrhoeal disease for which *V. cholerae* is responsible ranges from a completely asymptomatic state to severe diarrhoea. It is now recognized that vibrios actually produce many more asymptomatic infections and mild cases of diarrhoea than severe cases.

Mild cases of *V. cholerae*-induced diarrhoea have no distinguishing clinical features and can be recognized only by stool culture. Such cases are of epidemiological importance in that they maintain the microbe in the community, but they do not present a clinical problem, and are largely unrecognized and untreated.

The patients who seek medical attention are those with severe diarrhoea, from which the mortality is high in untreated cases. The symptoms and signs of the disease are entirely due to the loss of fluid and electrolytes from the small bowel. Classical vibrios and El Tor vibrios have been shown to produce an identical syndrome (Wallace et al., 1966).

These patients usually have an abrupt onset of effortless vomiting and watery diarrhoea that quickly assumes the characteristic "rice-water" appearance (colourless, with flecks of floating mucus), although it may occasionally be cream-coloured or even bloody. Soon after the onset of diarrhoea, the patient ceases to urinate and exhibits a moderate degree of thirst; later, cramps occur in the muscles of the extremities, and sometimes also in the external abdominal muscles. As diarrhoea continues, the patient's speech becomes hoarse and he sometimes develops aphonia. He gradually becomes weaker and eventually collapses. This progression of symptoms usually occurs within 5–12 hours of the onset of the disease.

On admission to hospital the patient is lethargic and weak, but conscious. His cheeks and eyes are sunken; skin turgor is markedly diminished; the skin over the fingers is shrivelled, and the neck veins

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are flat. The extremities are cold, often pulseless, and blood pressure is low or cannot be measured by the usual methods. There may be cyanosis of the fingertips, and of the tongue and lips. The pulse is rapid, if palpable at all, and heart sounds are faint. The abdomen is usually soft and not tender. The rectus abdominis muscle may be in spasm and give the false impression of abdominal guarding. Cramping of the hands and feet may be noted. The patient exudes a characteristic odour, owing to the rice-water stool which has usually soiled his clothes; the odour is of a sweet, fishy type, and not at all faecal in character.

Laboratory findings on admission (Carpenter et al., 1966) show evidence of: (1) marked saline depletion, with an elevation of the haematocrit (55–65) and plasma specific gravity (1.035–1.050); and (2) acidosis, with low blood pH (7.1–7.2) and low bicarbonate content (5–12 mEq/litre). Initially, the serum potassium level is normal or slightly elevated: it later decreases as the acidosis is corrected. The serum sodium and chloride contents are within normal limits, indicating the isotonic nature of the diarrhoea.

Patients with this clinical picture, if untreated, have a mortality rate of 60% or greater. With adequate treatment, however, mortality should be almost nil in all age groups.

The saline depletion and acidosis can be corrected within 2–3 hours of admission in adults and within 8 hours in children. By this time vomiting has ceased, and the patient feels well. Diarrhoea may continue, however, for another 2–6 days, with a total stool production of 1–60 litres, depending upon whether antibiotics are given for treatment. Provided that fluids are adequately replaced during this time the patient continues to feel well. Following the cessation of diarrhoea, which is self-limiting, the patient has no sequelae from the disease.

There are a few clinical features of cholera that are seen in children but not in adults: (1) the sensorium is usually altered to a greater degree; (2) seizures are occasionally seen, sometimes before and sometimes after treatment is begun; in some children they are due to marked hypoglycaemia, but the cause is not always known; and (3) fever is frequently seen in children, but rarely in adults.

In pregnant women with cholera, miscarriage is common; the rate was about 50% in one study (Hirschhorn et al., 1969). Placental retention may also be observed. However, cholera should not lead to increased maternal mortality.

Except for these problems unique to children and pregnant women, the only complications seen in cholera patients are due to inadequate therapy (see chapter 10). Acute renal failure, which used to be a dreaded complication of cholera, does not occur when adequate fluid therapy is provided.

The "clinical" cholera syndrome is not produced uniquely by cholera vibrios. Other organisms have been incriminated as a cause of this severe diarrhoeal state, notably non-agglutinable vibrios and certain strains of *Escherichia coli*, both of which colonize the small bowel. In some cases no recognized organisms can be found. The single distinguishing feature of this non-cholera syndrome is its short duration. In spite of identical clinical and biochemical features on admission, these patients usually have only a small amount of diarrhoea after admission. The reasons for this are not known at present.

REFERENCES

Carpenter, C. C. J. et al. (1966) *Bull. Johns Hopk. Hosp.*, **118**, 174
Hirschhorn, N. et al. (1969) *Lancet*, **1**, 1230
Wallace, C. K. et al. (1966) *Brit. med. J.*, **2**, 447

CHAPTER 10

MANAGEMENT OF CHOLERA IN ADULTS AND CHILDREN

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Effective treatment of cholera can reduce mortality in all ages to less than 1 % (Carpenter et al., 1966a; Gordon et al., 1964; Wallace et al., 1968). Such treatment is neither technically difficult nor expensive. The basis of adequate treatment is intravenous replacement of the water and electrolytes lost in the stool. Proper intravenous therapy alone with no other adjuncts to treatment can reduce mortality to practically nil (Carpenter et al., 1966a).

ADULTS

For the purpose of this discussion, "adults" may be considered to be individuals weighing more than 20 kg.

The composition of intravenous fluids

The replacement fluids are designed to replace stool losses. The composition of cholera stool is sufficiently predictable to allow a standard intravenous replacement fluid to be utilized for both rehydration and subsequent maintenance. The approximate composition of cholera stool, in mEq/litre, is as follows: Na^+, 135; K^+, 15; HCO_3^-, 40; and Cl^-, 100. Its osmolarity is about 290 mosm/litre. An adequate intravenous replacement solution should provide the following (in mEq/litre): sodium, 130–155; chloride, 90–110; and hydrogen carbonate or lactate, 28–52. Its osmolarity should be 250–290 mosm/litre. Intravenous potassium is not required in the treatment of adults (Carpenter et al., 1966b), but may be safely included up to 15 mEq/litre. Intravenous calcium and magnesium are not required.

Easily available standard intravenous fluids meet these needs when used in one of the following combinations:

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(1) Physiological saline, 8.9 g/litre (Carpenter et al., 1966b), and sodium hydrogen carbonate in isotonic solution (14.0 g/litre). These may be administered in a ratio of 2 units of saline to 1 unit of hydrogen carbonate; "units" of 3 bottles in this 2: 1 ratio are repeated throughout the course of fluid replacement. This combination provides (mEq/litre): sodium, 154; chloride, 103; and hydrogen carbonate, 51.

(2) An equally useful combination that avoids some of the difficulties involved in sterilizing, storing, and mixing hydrogen carbonate solutions consists of physiological saline and sodium lactate in 1/6 molar solution (18.7 g/litre). This combination is also given in the proportion of 2 parts of saline to 1 part of sodium lactate.

(3) Special single-solution fluids that meet the requirements listed above may also be prepared. One such is "5:4:1 solution" (Greenough et al., 1964), which contains 5 g of sodium chloride, 4 g of sodium hydrogen carbonate, and 1 g of potassium chloride per litre.

(4) A fourth (commercially available) solution that can be used as a single replacement fluid is Ringer's lactate (Hartmann's Solution for Injection, BPC & USP). This solution provides approximately 4 mEq of Ca^{2+}, 131 mEq of Na^{+}, 5 mEq of K^{+}, 111 mEq of Cl^{-}, and the equivalent of 29 mEq of HCO_3^{-} per litre. This provides less hydrogen carbonate than the combination above, but the amount provided is sufficient to replace a large portion of the hydrogen carbonate lost in the stool and to prevent serious metabolic acidosis.

The importance of adequate replacement of base loss (as hydrogen carbonate or lactate) cannot be over-emphasized. During rapid diarrhoea the stool losses of hydrogen carbonate exceed the renal mechanism of hydrogen carbonate production even if urine output is restored. If hydrogen carbonate is not replaced severe acidosis results.

Management

Fluid replacement is conveniently divided into two phases. The first, rehydration, is an attempt to replace the water, sodium, and hydrogen carbonate lost up to the time of admission. At the end of this phase the patient may continue to have rapid stool loss but will no longer be in serious acidosis or shock. The second phase, maintenance, is the period during which continuing stool losses are replaced as they occur.

Rehydration. Rehydration must be carried out with intravenous fluids and should be accomplished as rapidly as possible, with the goal of restoring normal hydration and acid-base balance within 2 hours after admission. In severely ill patients, fluid should be infused as rapidly as possible (1000 ml per 15 minutes) until an easily palpable radial pulse is present. The use of a large-bore (No. 18) needle that will permit

a rapid flow is important. Following this initial rapid infusion (usually 1000–2000 ml), rehydration is completed at a somewhat slower rate (1000 ml per 30–45 minutes) until the patient is in a state of normal hydration as judged by the criteria given below. Patients with severe dehydration and cardiovascular collapse have a fluid deficit equal to about 10% of their body weight. Consequently, complete replacement of the initial volume deficit in a seriously ill, 50-kg adult may necessitate the administration of as much as 5 litres of fluid.

Maintenance. This is accomplished by replacing measured stool output after rehydration with intravenous fluids (or with oral glucose containing electrolyte solution; Nalin et al., 1968) until diarrhoea ceases. Accurate stool measurement is very important and is easily accomplished by use of a " cholera cot " or bed (see the figure on page 74) that has been altered so as to provide a hole about 23 cm (9 in) in diameter beneath the patient's buttocks. A rubber sheet with a central sleeve passing through the hole covers the bed. All stool is easily passed though this hole and collected for measurement in a bucket beneath the bed. Urine should be collected separately from stool. The bucket may be calibrated in litres to facilitate measurement of stool losses. All fluid intake and stool output should be recorded on a bedside chart.

When intravenous fluids are used for maintenance therapy stool losses are replaced with equal volumes of intravenous fluid.

When oral maintenance fluids are given, the replacement depends on the composition of the oral fluid used. (Details of oral maintenance therapy are given in chapter 11.) One oral solution that has been shown to be effective has the following composition: sodium, 90 mEq/litre; chloride, 60 mEq/litre; hydrogen carbonate, 30 mEq/litre; and glucose, 120 mmol/litre. This solution may be prepared by dissolving, in 1 litre of water, 3.50 g of sodium chloride, 2.52 g of sodium hydrogen carbonate, and 21.6 g of glucose. This fluid is used to replace stool losses in a ratio of 1½ volumes of oral fluid replacement for every volume of stool produced.

The adequacy of fluid replacement may be assessed by the following methods:

(1) Return of blood pressure and pulse to normal. When adequate rehydration is achieved the pulse rate will almost always be below 100 per minute and the pulse volume will be full. Bedside evaluation of pulse is a simple and useful means of assessing fluid replacement (Wallace et al., 1964).

(2) Return of skin turgor to normal. If skin turgor has returned to normal and pulse remains rapid and weak, other causes of shock could be considered—e.g., sepsis.

(3) Return of a feeling of comfort to the patient, with the absence of cyanosis, muscular cramps, and nausea or vomiting. Vomiting will not usually last longer than 3 hours after admission to hospital in an adequately treated patient.

(4) Return of urine output to normal. This usually occurs within 12–24 hours after initial rehydration.

(5) Return of plasma specific gravity (Gp) to normal (1.024–1.027). The procedure for determing Gp is described in the Annex.

Adjuncts to therapy

The following are useful adjuncts to cholera therapy, but they are in no way substitutes for the intravenous replacement of water, sodium chloride, and hydrogen carbonate or lactate, which is of primary importance.

Tetracycline. Tetracycline should be administered at a dosage of 500 mg by mouth every 6 hours for 48 hours (Carpenter et al., 1966c; Wallace et al., 1968). This will:

(*a*) reduce the duration of diarrhoea by 50%, to an average of about 2 days;

(*b*) reduce the volume of diarrhoea after initiation of treatment by 60%, to about 6–8 litres per patient in persons with severe disease;

(*c*) reduce the duration of vibrio excretion to an average of 1.1 days and a maximum of 48 hours.

This will effectively lessen the expense of treatment, the duration of hospitalization, and the complications of prolonged intravenous maintenance. Because of the likelihood of vomiting, tetracycline should not be administered until the patient is fully rehydrated with no vomiting. This usually takes about 3 hours after entry. If tetracycline is not available, other useful antibacterial agents, which are only slightly less effective, are furazolidone (Pierce et al., 1968a) and chloramphenicol (Wallace et al., 1968). The former is given in a 100-mg dose every 6 hours and the latter in a dose of 500 mg every 6 hours, each for a total of 72 hours.

Oral fluids. Patients receiving intravenous maintenance therapy should be allowed moderate amounts of oral water, after nausea and vomiting have ceased. A portion of oral water is absorbed, as are orally administered potassium and hydrogen carbonate (Phillips, 1965). Such oral water is essential as a replacement for insensible losses and urinary output occurring during therapy. Patients receiving oral maintenance therapy (as outlined previously) do not require any additional water, since adequate "free" water is supplied in the electrolyte solution.

Oral potassium supplementation. Orally administered potassium is absorbed by cholera patients even during acute diarrhoea. If potassium is not included in the intravenous solutions it should be replaced orally. Green coconut water provides a cheap source of oral potassium (Carpenter et al., 1963), and about 170 ml given by mouth for each litre of cholera stool will replace nearly all potassium losses. Alternatively, a satisfactory solution can be made from 100 g each of potassium acetate, potassium citrate, and potassium hydrogen carbonate dissolved together in 1 litre of water; 10-ml doses of this solution suitably diluted and given 3 times a day with food will provide adequate replacement of potassium losses (Watten et al., 1960).

There are no other adjuncts of confirmed value in the treatment of cholera.

Diet

There is no good reason to limit the diet during cholera unless a patient is vomiting. Patients should be allowed to eat a normal diet as soon as they so desire. Resumption of full diet need not await the termination of diarrhoea.

Termination of treatment

Fluid replacement should continue until diarrhoea ceases. Tetracycline therapy should continue until the full course is completed even though diarrhoea may cease before this time. If these guide-lines are followed, bacteriological relapse or recurrence of diarrhoea are unlikely (Carpenter et al., 1966c; Pierce et al., 1968a). The patient may be discharged within 24 hours of the cessation of diarrhoea and the completion of antibiotic therapy. Patients have only rarely been observed to excrete vibrios more than 48 hours after the initiation of tetracycline therapy in the dosage recommended; however, with lower tetracycline dosage prolonged excretion occurs more frequently (Carpenter et al., 1966c). During epidemics, when hospital beds are overcrowded, it is probably not necessary to prolong hospitalization to ensure by culture methods that the patient is no longer excreting vibrios. Hospitalization may thus be limited to a duration of about 3 days.

Complications

A common complication is that of pyrogen reactions resulting from the administration of incorrectly prepared fluids or the repeated use of the same rubber tubing for administering parenteral fluids. This can be avoided by using pyrogen-free fluids and disposable administration sets.

All serious complications of cholera in adults are prevented by appropriate fluid, electrolyte, and hydrogen carbonate replacement. If therapy is inadequate the following may occur:

(1) persistence or recurrence of dehydration, hypovolaemia, and shock owing to inadequate volume replacement (if a cholera patient does not appear to be responding adequately to therapy, the first priority is a careful assessment of the adequacy of fluid replacement, using the methods described);

(2) persistence of nausea or vomiting, which may be due to uncorrected acidosis or hypovolaemia;

(3) renal failure, owing to prolonged or repeated episodes of hypotension as a result of inadequate fluid replacement;

(4) acute pulmonary oedema, due to over-replacement of intravenous electrolyte solutions in the presence of continuing uncorrected metabolic acidosis;

(5) hypokalaemia (potassium deficiency is rarely symptomatic in adults, but in children it may produce cardiac arrythmias, abdominal distension, and weakness); and

(6) overhydration (more easily produced in children or infants than in adults; distended neck veins and a slow full pulse usually precede frank cardiac failure).

A further danger is that of incorrect diagnosis. During cholera epidemics other serious illnesses presenting diarrhoea may be diagnosed clinically as "cholera". Such diseases may include meningitis, typhoid fever, various bacteraemias, and heavy-metal poisoning. These obviously require specialized treatment and will not respond to the therapy for cholera.

CHILDREN

With less than optimum therapy, cholera mortality is greater in children than adults, particularly in those under 5 years of age. Mortality increases as age decreases for the following reasons:

(1) severe dehydration and its consequences can occur more rapidly in smaller children;

(2) water and electrolyte replacement are technically more difficult in small children, and errors in the rate of administration, volume, or composition of intravenous fluid cause serious morbidity or mortality; and

(3) children develop complications of electrolyte imbalance and acidosis that are seldom seen in adults, including hypoglycaemia, convulsions, cerebral oedema, and paralytic ileus.

With treatment methods that adhere to established concepts of water and electrolyte replacement in children, mortality from cholera can be essentially nil (Mahalanabis et al., unpublished data). These concepts as applied to paediatric cholera are discussed in the following sections.

General requirements for fluid replacement

(1) All water and electrolyte replacement must be based on calculated requirements, using objective measurements such as body weight and measured stool output. Careful records of fluid administration, body weight, and stool output must be maintained. Estimation of body weight or stool output by guessing will frequently lead to errors, with serious consequences for the patient.

(2) Specific supplies for intravenous fluid replacement are required, including short small-gauge needles (No. 22–24) and paediatric scalp vein sets.

(3) Intravenous fluid replacement should avoid rapid changes in the blood pH, osmolarity, or volume, as these may be associated with serious complications including grand mal convulsions and pulmonary oedema. A common error, with serious consequences, is the over-rapid administration of an excessive volume of intravenous fluid.

(4) The composition of intravenous replacement fluid should be such as to replace the water and electrolyte deficits present on admission and occurring thereafter (Mahalanabis et al., 1970). Initial rehydration requires an isotonic electrolyte solution to correct the isotonic dehydration. Replacement of continuing stool losses requires a solution with lower sodium content because of the lower sodium content of stool in paediatric cholera (Mahalanabis et al., 1970). The average composition of the stool in childhood cholera is as follows (mEq/litre): Na^+, 105; K^+, 25; Cl^-, 90; and HCO_3^-, 30. Its average osmolarity is 290 mosm/litre.

(5) If serious over-hydration or under-hydration is to be avoided, water and electrolyte requirements must be reassessed at frequent intervals (every 4 hours) during active diarrhoea. Changes in the rate of stool loss must be accompanied by changes in fluid administration. Bedside physical examination is of great value, specifically the evaluation of pulse rate and volume, skin turgor, level of consciousness, facial oedema, and lung fields, and the measurement of blood pressure and of urine production. An accurate body weight determination is essential and can be accomplished with an inexpensive pan balance, which should be located in the paediatric treatment area.

(6) Intravenous fluids should contain some glucose to prevent severe hypoglycaemic coma or convulsions, which may occur in children with diarrhoea (Hirschhorn et al., 1966).

(7) Children should be treated in an area separate from adults, equipped with appropriate supplies, and staffed with persons trained in paediatric nursing and treatment.

Initial treatment

Cholera in children causes isotonic dehydration (Mahalanabis et al., 1970). The associated volume deficit rarely exceeds 11% of the body weight and may be roughly estimated as follows:

(1) mild dehydration (slightly decreased skin turgor, tachycardia, normal sensorium, not in shock): about 5% fluid deficit;

(2) moderate dehydration (definitely decreased skin turgor, tachycardia, hypotension, but normal sensorium): about 8% fluid deficit:

(3) severe dehydration (the above signs together with cyanosis, stupor or coma, sunken eyes or fontanelles, absence of radial pulse, definite shock): about 10–11% fluid deficit.

Upon admission, the body weight should be measured and recorded and the fluid deficit estimated on the basis of physical findings as described above. For example:

Body weight	8.35 kg
Estimated deficit	10%
Initial fluid requirement	835 ml

The initial fluid requirement necessary for rehydration is given as follows: (1) first hour; 2–3% of body weight or 20–30 ml per kg of body weight (167–250 ml in the above example); and (2) second to eighth hours, the remainder given more slowly (585–667 ml in the above example).

Ringer's lactate solution is satisfactory for initial rehydration. I this is not available, one of the following can be used, provided the components are mixed in a single bottle: (1) 2 parts of normal saline to 1 part of 1/6 molar sodium lactate; (2) 2 parts of normal saline to 1 part of 1.3% sodium hydrogen carbonate. These solutions contain 20 mEq/litre more sodium than is present in Ringer's lactate, and if they are used, the volume given should be about 15% less than that recommended above for Ringer's lactate.

When rehydration is completed and the child's hydration is judged to be normal by careful physical examination, he should be weighed again. This "normal" weight may be used for reference purposes in determining further fluid requirements during the remainder of his illness. Alternatively, the weight on admission plus the estimated fluid deficit on admission may be used for reference as the normal weight.

Weight after rehydration should never exceed the admission weight plus 10%; if this occurs, excessive fluid has been given.

Replacement of continuing losses

Children should be treated on metabolic beds that allow the liquid stool to be collected in a calibrated container beneath the bed. The amount of fluid necessary to replace continuing losses is determined by stool output and by respiratory, skin and urinary losses of water.

In female patients, or in situations where accurate measurement of stool output may be difficult, changes in body weight from the normal hydrated state may be used as a measurement of stool output. Measured stool losses are replaced on a volume-for-volume basis by a solution prepared as follows: to an 0.45% solution of sodium chloride in 2.5% or 5% dextrose (which is commercially available), sodium hydrogen carbonate in the amount of 32 mEq/litre is added immediately before administration. This solution contains 106 mEq/litre of Na^+, 74 mEq/litre of Cl^-, and 32 mEq/litre of HCO_3^-, closely approximating (except for potassium) the composition of cholera stool in children. Alternatively, Ringer's lactate can be used for replacement therapy, in which case intravenous replacement should be approximately 25% less than stool output.

The water necessary to replace evaporative and urinary losses is estimated at 120–130 ml per kg of body weight per day; it is administered by mouth as soon as nausea and vomiting cease (usually within 6–8 hours of admission), or intravenously in the form of 1/5 normal saline in 5% glucose if oral intake cannot be initiated within 8 hours of admission. When giving water by mouth, 5% glucose should be added to prevent hypoglycaemia.

An alternative form of maintenance therapy is the administration of fluids intraperitoneally. This may be particularly useful when prolonged intravenous therapy is technically difficult. Ringer's lactate in approximately 500-ml amounts may be given through a No. 18 needle placed in the midline, just below the umbilicus. The fluid can be given in about 10 minutes, and the needle removed. This can be repeated when necessary, as determined by the stool output. Liberal amounts of glucose water should be given by mouth, as mentioned above. The majority (about 90%) of small children will absorb the fluid rapidly enough to maintain normal hydration. Maintenance of normal blood pressure, radial pulse, and sensorium during therapy is evidence of adequate fluid adsorption. However, the few children with high stool outputs (about 9 ml per kg of body weight per hour or greater) will require additional intravenous fluids, since peritoneal adsorption is not rapid enough to replace such large stool losses.

The adequacy of fluid replacement should be evaluated at frequent intervals (every 4 hours) by physical examination and by the determination of urine output, body weight, and, if necessary, plasma specific gravity.

Adjuncts to therapy

Tetracycline should be given in a dosage of 50 mg per kg of body weight per day, divided into 4 doses administered at intervals of 6 hours. A liquid suspension is desirable for this purpose. The first dose should be given about 3 hours after admission and therapy should continue for 48 hours.

Potassium replacement is important and may be adequately accomplished by mouth. The dose should be 4 mEq per kg of body weight per day, divided into 4 doses given every 6 hours. Either commercially available potassium replacement mixtures or the solution described in the section on adult therapy may be used.

Diet

A normal diet for age may be given as soon as the child is free from nausea and vomiting and is able to eat. A normal diet need not be withheld until diarrhoea ceases.

Termination of therapy

If tetracycline is given, diarrhoea should cease within 36 hours. Parenteral fluid replacement should be continued until diarrhoea ceases. The child may be discharged when diarrhoea has ceased and tetracycline treatment is completed.

Complications

Convulsions. Convulsions are usually due to the administration of intravenous fluid at too great a speed and in excessive volume. If the above guide-lines are followed carefully, the incidence of convulsions will be greatly reduced. On rare occasions, convulsions may occur prior to the initiation of treatment.

Hypoglycaemia. This is a consequence of the exhaustion of glycogen stores and may occur rapidly in children (Hirschhorn et al., 1966). It may be manifest as prolonged stupor or convulsions. It may be prevented by the inclusion of glucose in the intravenous fluids and drinking-water. If convulsions or prolonged stupor should occur, 25 ml of 50% glucose should be given intravenously; if hypoglycaemia is present, this will produce a rapid improvement in the sensorium of the child.

Over-hydration. This may be manifest as generalized oedema, pulmonary oedema, congestive heart failure, altered consciousness, and weight gain exceeding the admission weight plus the estimated deficit.

REFERENCES

Carpenter, C. C. J. et al. (1963) *Bull. Calcutta Sch. trop. Med.*, **12**, 20
Carpenter, C. C. J. et al. (1966a) *Bull. Johns Hopk. Hosp.*, **118**, 174
Carpenter, C. C. J. et al. (1966b) *Bull. Johns Hopk. Hosp.*, **118**, 197
Carpenter, C. C. J. et al. (1966c) *Bull. Johns Hopk. Hosp.*, **118**, 216
Gordon, R. et al. (1964) *Pak. med. J.*, **8**, 10
Greenough, W. B. et al. (1964) *Lancet*, **1**, 355
Hirschhorn, N. et al. (1966) *Lancet*, **2**, 128
Mahalanabis, D. et al. (1970) *Pediatrics* (in press)
Nalin, D. R. et al. (1968) *Lancet*, **2**, 370
Phillips, R. A. (1965) In: *Proceedings of the Cholera Research Symposium, Honolulu*, Washington, D. C., US Government Printing Office (PHS Publication No. 1328)
Pierce, N. F. et al. (1968a) *Brit. med. J.*, **3**, 277
Wallace, C. K. et al. (1964) *Bull. Wld Hlth Org.*, **30**, 795
Wallace, C. K. et al. (1968) *Bull. Wld Hlth Org.*, **39**, 239
Watten, R. H. et al. (1960) *Lancet*, **2**, 199

Annex

PROCEDURE FOR DETERMINATION OF PLASMA SPECIFIC GRAVITY

The determination of plasma specific gravity (Gp) is a simple and useful procedure for assessing the severity of dehydration and for confirming the adequacy of fluid replacement.[1] Sufficient plasma is obtained by centrifugation or sedimentation of about 3 ml of heparinized or clotted venous blood. Copper sulfate is prepared in solutions of graded specific gravity ranging from 1.020 to 1.050 in gradations of 0.001 or 0.002 units. These solutions are prepared from a stock solution of copper sulfate (gravity 1.1000) which contains 159.63 g of analytical-reagent-grade copper sulfate ($CuSO_4 . 5H_2O$) dissolved in distilled water to make a volume of 1 litre. Graded solutions are made by diluting to 100 ml a volume of stock solution 1 ml less than the last 2 figures of the desired specific gravity; for example, 25 ml of stock solution diluted to 100 ml gives a solution having a specific gravity of 1.026, 30 ml of stock solution diluted to 100 ml gives a solution having a specific gravity of 1.031, etc. The solutions thus prepared are stored in screw-top bottles of about 120 ml capacity.

To determine Gp, a drop of plasma is allowed to fall from a dropper held about 2 cm above the surface of the copper sulfate solution. The drop must break

[1] Phillips, R. A. (1950) *J. biol. Chem.*, **183**, 305.

completely through the surface of the solution. If it subsequently falls steadily to the bottom, it is of greater specific gravity than the solution and the procedure is repeated with a solution of higher specific gravity. If, after breaking through the surface of the solution, the drop stops falling and starts to rise, it is of lower specific gravity. If it hangs motionless for several seconds in the middle of the solution it is of the same specific gravity as the solution. After about 20 seconds all drops will become saturated with copper sulfate and fall to the bottom of the container.

Normal Gp is 1.024–1.027. In severe dehydration it may be 1.035–1.050. If the Gp is less than 1.030 in a patient with continuing hypotension or other evidence of shock, it should be assumed that shock is due to a cause other than dehydration with hypovolaemia.

CHAPTER 11

ORAL OR NASOGASTRIC THERAPY FOR CHOLERA*

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INTRODUCTION

Oral maintenance therapy for cholera was introduced by the Pakistan-SEATO Cholera Research Laboratory in 1968 (Nalin et al., 1968). A clinical trial in 1968-69 during a large epidemic showed that the method was practical in a cottage hospital managed by a small staff. Since the original report on adults and a confirmatory report from another laboratory (Pierce et al., 1968), the solution administered to the patients has been modified for use in all age-groups. The advantages of oral therapy are (1) it reduces the intravenous fluid needs of severe cases by 80%; (2) mild or moderately severe cases on oral therapy need no intravenous fluid; (3) it reduces the cost of therapy, since the ingredients are cheap and available in all endemic areas, and they do not need to be sterile.

METHOD

Patients are placed on a cholera cot (see Fig.). Stools, urine, and vomitus are collected and measured separately. Patients with a clinical diagnosis of cholera who are in shock due to dehydration receive initial rehydration with a standard intravenous solution for cholera.[1]

When the pulse and blood pressure return to normal, the maintenance phase is started. The solution is warmed and delivered to the patients at 40–45°C. Most patients drink the solution, but it can also

* The studies on which this chapter is based were supported in part by Research Agreement No. 196802 between the National Institutes of Health, Bethesda, Md., USA, and the Pakistan-SEATO Cholera Research Laboratory, Dacca, East Pakistan.

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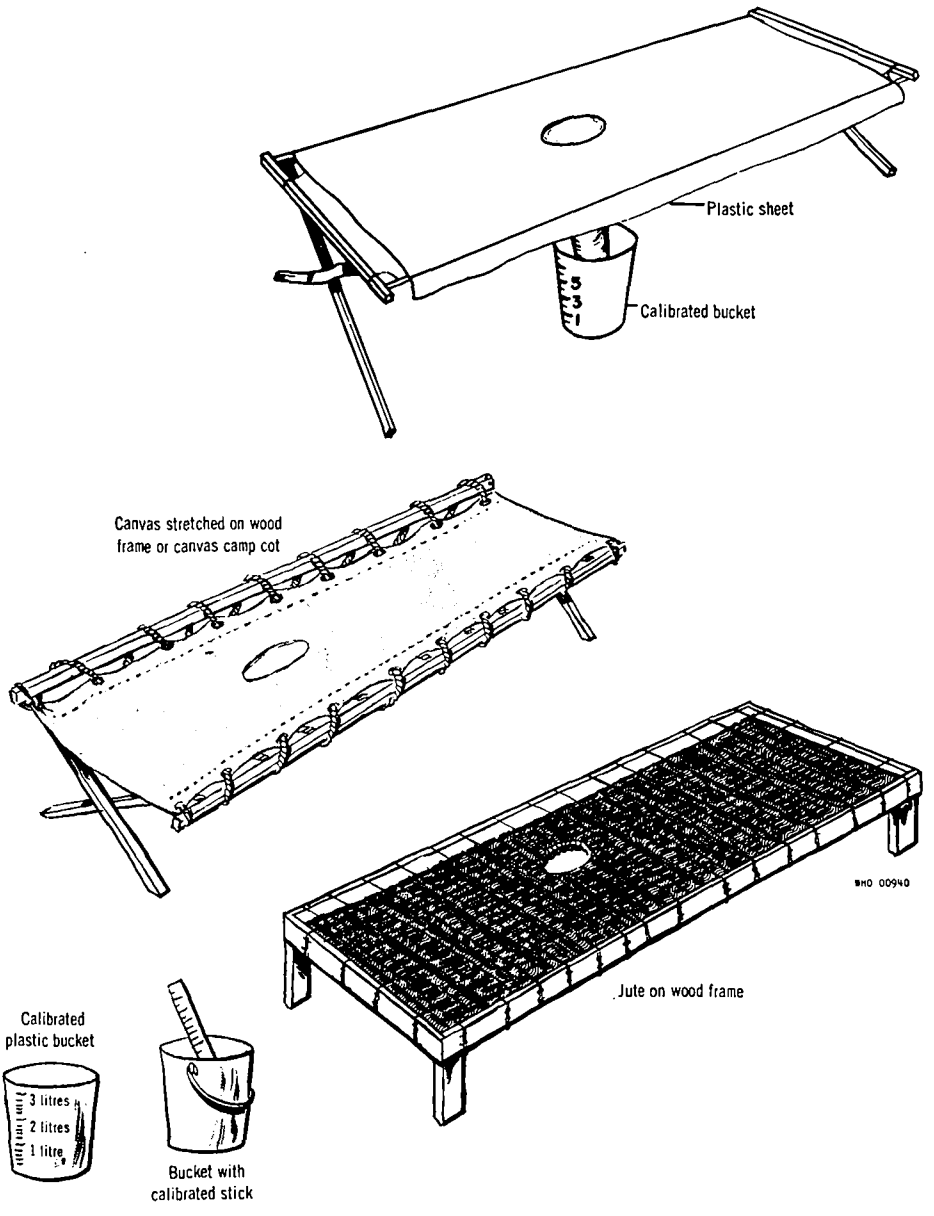
[1] Our standard intravenous solution for cholera contains, per litre of water, 5 g of NaCl, 4 g of $NaHCO_3$ and 1 g of KCl.

CHOLERA COT AND COLLECTING BUCKET



Bucket is placed under hole in cot to collect stool

be delivered via intravenous-type tubing connecting an inverted bottle of solution with a nasogastric tube. Nasogastric tubes allow patients to sleep and facilitate nursing supervision of intake; they are often necessary for younger children.[1]

During the first 6 hours the solution is usually drunk (or infused) at the rate of 750 ml/h for adults and 250 ml/h for children. The initial rate is increased or decreased to match initial diarrhoea volumes. After the first 6 hours, the volume of maintenance solution given during any subsequent 6-hour period equals the diarrhoea volume of the preceding 6-hour period. If the diarrhoea volume significantly exceeds the intake of the solution, the difference is made up with intravenous therapy.

The composition of the maintenance solution currently in use for all age-groups is given in the accompanying table. Patients treated during 1968-69 received the same solution, except that the potassium concentration was only 15 mEq/litre, which is too low for children with cholera.

COMPOSITION OF MAINTENANCE SOLUTION*

	Millimoles per litre
Glucose	110
NaCl	72
$NaHCO_3$	48
KCl	25

* Oral solution ingredients are pre-weighed in plastic bags at the field hospital in amounts sufficient to make 15 litres of solution when added to drinking-water. Concentrations are given in millimoles per litre because the purity of locally available salts varies and should be checked.

Patients are given nothing by mouth except the oral solution and tetracycline. Attendant relatives assist staff in checking that the amount of solution given is drunk in the required time. Tetracycline is given for 5 days in two dose regimens: 125 mg every 6 hours for children, and 250 mg every 6 hours for adults. Oral therapy is stopped when the diarrhoea rate is less than 75 ml/h in adults, or 30 ml/h in children. The diagnosis of cholera is confirmed by culture of rectal swabs.

[1] Patients with complications, as well as very young and very old patients, are generally not treated with oral solution, since management of these cases poses special problems.

After each 6-hour intake and output period, the net gut balance is calculated by subtracting the combined stool and vomitus volume from the oral (or intragastric) intake. When the net gut balance is positive, no intravenous fluid is needed. If the net gut balance during any 6-hour period is negative, the fluid deficit is replaced intravenously.

A history of vomiting is obtained in 90% of all cholera patients who arrive at our hospital in shock. Among adult patients with severe cholera, 70% vomit after admission and 60% of these vomit a total of less than 1.5 litres. Another 25% vomit more than 1500 ml but less than 3500 ml. Only 2% of patients vomit more than 5 litres.

Since vomiting occurs primarily in the first hours after admission, and since severely ill patients are receiving 1000–1500 ml of oral fluid per hour, the loss of several litres of vomitus usually does not prevent patients from achieving positive gut balance after admission. Positive gut balance is achieved in 80% of cases within the first 6 hours of oral maintenance, and in 90% within 12 hours. When positive gut balance has been achieved, intravenous fluid can be discontinued. Less than 5% of patients with positive gut balance will revert to negative balance and require more intravenous fluid.

CONCLUSIONS

Our maintenance solution for cholera is absorbed well and maintains a positive water and electrolyte balance. The use of one formula for all age-groups facilitates the management of large epidemics. Our experience with over 1000 cases confirms the practicability and benefits of the method on a massive scale. If our maintenance solution is used, a limited supply of intravenous fluid will serve for the treatment of more than 5 times the usual number of cholera patients. Oral maintenance can be life-saving, and can reduce the cost of therapy in epidemic areas where intravenous fluids are scarce.

REFERENCES

Nalin, D. R. et al. (1968) *Lancet*, **2**, 370–372.
Pierce, N. F. et al. (1968) *Indian J. med. Res.*, **56**, 640

CHAPTER 12

IMMUNITY IN CHOLERA

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INTRODUCTION

In cholera, the pathogenic organisms multiply in the intestinal lumen but do not cross the intestinal epithelium to produce invasive infection. The effects on the host that represent clinical cholera are mediated entirely by toxic substances that are elaborated by *V. cholerae* within the intestine and act upon the intestinal mucosa and possibly in immediately subjacent tissues. It seems reasonable to suppose that clinical cholera would not occur if the organism failed to multiply and produce toxin or if the host tissues were not susceptible to the toxin generated. Antibodies can be produced both against the organisms themselves and against their toxic products. For purposes of discussion, therefore, immunity to cholera can be divided into antibacterial immunity and antitoxic immunity according to whether antibody is directed against the function of the organism or against the action of its toxins. Although knowledge of antibacterial immunity is far from complete, well-controlled field trials of vaccines have unequivocally proved its existence at a practical level. The status of antitoxic immunity as a means of preventing ahe clinical manifestations of cholera is less clear, and is one of the most ftctive areas of cholera research today.

ANTIBACTERIAL IMMUNITY

It is now clear that certain chemically-killed, conventional cholera vaccines, when administered subcutaneously, have afforded significant protection to human subjects living in endemic areas. These trials have also indicated that the degree of effectiveness and the duration of immunity may be somewhat limited, particularly in infants and children. The

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development of antitoxic immunity by conventional vaccines has apparently not contributed to the favourable field trial results, since one of the most effective vaccines used in the Dacca trials of 1963–1964 was shown not to induce antitoxic immunity. Furthermore, other effective vaccines have been found to produce very little or no antitoxic antibody even when used in the hyperimmunization of animals. The association of protective activity with the cellular antigens was further supported by the findings of the Dacca field trial of 1968, when a purified somatic antigen derived from Inaba cells was shown to produce highly significant protection in the Inaba epidemic. Several studies have shown that if vaccine-induced immunity is overcome to the point where symptomatic cholera occurs, the disease is indistinguishable in severity and duration from that in persons who have received a placebo vaccine affording no immunity.

Most field trials in recent times have employed bivalent vaccines containing organisms of both Ogawa and Inaba serotypes. Since there is some degree of antigenic cross-reactivity, it has not been possible to evaluate the relative contribution to immunization supplied by organisms of the two serotypes. Monovalent vaccines were used for the first time in the East Pakistan field trial of 1968–1969, when children up to 15 years of age were immunized. In a subsequent cholera epidemic caused predominantly by Inaba strains of the classical biotype, the Inaba monovalent vaccine was highly protective but the Ogawa vaccine gave no protection in children 5 years of age and under, and little if any protection in older children. It seems therefore that normal doses of Ogawa vaccine afford little cross-protection against Inaba cholera, but up to now there is no evidence from field experience to indicate whether Inaba vaccines may be effective against cholera caused by Ogawa strains.

The Philippine field trial of 1964 demonstrated that vaccines of both classical and El Tor strains can protect against cholera caused by Ogawa organisms of the El Tor biotype. In addition, since the purified Inaba antigen that was found to be protective in the East Pakistan field trial of 1968–1969 was derived from an Inaba El Tor strain, there is now evidence that Inaba El Tor organisms contain antigens protective against Inaba cholera caused by the classical biotype. Circumstances have not yet permitted the trial of El Tor vaccines against classical Ogawa infection or classical vaccines against El Tor Inaba infection. In experiments with mice, however, vaccines of different biotypes appear to have similar protective activity.

A serological survey in East Pakistan, where cholera has been endemic for many generations, showed that the geometric mean serum vibriocidal antibody titres of the population increased with age and that there was a good relationship between the decline in the cholera case rate in the

various age groups and the rise in antibody level. It was also found in related studies in the same area that the frequency of clinical cholera among familial contacts of cholera victims was lowest among individuals with high vibriocidal antibody levels and progressively increased among persons with lower titres. These observations suggest a possible correlation between serum vibriocidal antibody titres and human protection, at least when the antigenic stimulation is induced by organisms that are homologous with the epidemic strains. The existence of such a correlation has been further supported by the finding in the 1968 field trial in Dacca that, when children were immunized with two doses of cholera vaccine, the geometric mean vibriocidal titre of the group was raised to approximately that of the normal adult population and the case rate fell to approximately that previously seen in unimmunized adults. Such a serological test would seem to have considerable usefulness in evaluating responses to antigens under the many conditions where field trials are not feasible. Although serum vibriocidal antibody titres have shown good correlations with case rates in certain specific field studies, their suitability for unrestricted use as an index of probable immunity is questionable. In East Pakistan, it was found that children with anti-Inaba vibriocidal antibody arising from immunization with Ogawa monovalent vaccine were not as well protected from Inaba cholera as children who developed lower vibriocidal titres following vaccination with an Inaba monovalent vaccine.

In appraising the significance of circulating vibriocidal antibody titres, it is important to recognize that antibodies producing vibriocidal effects are commonly found in the sera of individuals in most areas of the world, even where cholera has not occurred for generations. While these titres tend to be relatively low, there is sufficient overlap between the titres found in non-endemic areas and those found in endemic areas to make it unlikely that serological studies would be of use in detecting the invasion by cholera of new territory, or in defining the boundaries of endemic areas.

Although the parenteral administration of vaccines has been shown both to stimulate antibody formation and to reduce the frequency of clinical infection, the mechanism of intestinal immunity is still unknown. In both human subjects and various animal models, symptomatic infection appears to be typified by close association of the vibrios with the intestinal mucosa and their appearance in the crypts of Lieberkuhn. Freter, using organisms injected into tied-off segments of rabbit ileum, has demonstrated this mucosal adherence, and has shown that it can be inhibited by the introduction of immune serum directly into the ileal segments or by the active immunization of rabbits with cholera vaccine. These experiments indicate that antibodies to somatic antigens are respon-

sible for this inhibition, and that both antibodies in serum and factors (antibodies?) present in the intestine following active immunization are effective. No data are yet available to suggest the mechanism whereby this adherence of vibrios to the intestinal mucosa is inhibited, but where it has been prevented no fluid accumulation has occurred.

There has been much speculation concerning the immune forces at work in the intestine, and the existence of specific copro-antibody has been well documented. With the recognition of secretory IgA as an immunoglobulin that is characteristic of mucosal surfaces, it was logical to postulate a relationship between copro-antibody activity and IgA. Studies using specific antiglobulin antisera have now demonstrated that intestinal fluids contain appreciable quantities of all the three major classes of human immunoglobulins (IgA, IgG and IgM). It has also been demonstrated, through a comparison of intestinal fluids obtained from acute and convalescent cholera cases, that antibody-like activity is increased in both the IgA and IgG components of intestinal fluids from convalescent subjects. Without denying a possible functional role for IgA, it appears that both IgG and IgM must now also be included in any consideration of intestinal immunity to cholera.

Regardless of what the mechanisms of intestinal immunity may be, the fact remains that certain vaccines have induced a significant degree of protection even when given as single parenteral doses. In comparative studies of the vaccines used in the field trials in the Philippines and in East Pakistan, which suffered epidemics of Ogawa and Inaba cholera respectively, the mouse protection test of Feeley and Pittman provided some indication of vaccine efficacy.

The effects of various vaccine dosages and schedules upon cholera immunity have been difficult to establish by field trial techniques. In the Philippine field trial of 1966, no significant differences could be detected between groups of persons receiving one or two doses of a vaccine containing 8000 million organisms, or one dose of a vaccine containing twice as much antigen. However, a study in East Pakistan among children from 3 months to 14 years of age demonstrated that a single dose of a commercial vaccine of "average" mouse-protective potency produced little protection in the group under 5 years of age but provided significant protection in older children. Two doses given at approximately a 4-week interval provided significant protection in both age groups. This lack of responsiveness among younger children may be related both to comparative immunological immaturity and to a lack of opportunity for the prior environmental antigen contact that would provide a naturally acquired primary antigenic stimulus.

In areas where cholera is endemic, prior contact with cholera antigens is an unavoidable problem that complicates the evaluation of immunity

resulting from vaccination. This may be overcome to some extent by carrying out vaccination studies in populations where cholera is not endemic, but under such circumstances results may be judged only by serological responses. Although definitive proof of the link between vibriocidal antibody and human protection is still lacking, the correlation now appears to be sufficiently well-founded to make results obtained with human subjects relevant to the problems of cholera immunity. Studies in children 6–12 months old have been carried out in a region of East Pakistan where cholera was not endemic. Children of this age have lost any significant maternally derived antibody and are likely to have little naturally acquired antibody, from contact either with cholera antigens or with other related antigens in their environment. In these very young children, a single dose of cholera vaccine produced only a slight rise in vibriocidal titre but 2 doses administered at intervals of 1 or 4 weeks produced a good antibody response, the 4-week interval being somewhat more successful. When the groups were re-immunized after 6 months, all showed very good responses. These children apparently experienced a true primary response to the first dose of vaccine, and a second dose given later (particularly after 4 weeks or 6 months) elicited a greatly enhanced "booster" response.

A similar experiment with adults in the United States of America produced quite different results. A single dose of vaccine produced a very considerable response, even in persons whose vibriocidal titres prior to immunization were not detectable. A group receiving a second dose a week later had somewhat improved titres, but a group receiving a second dose 4 weeks later showed little additional stimulation except in the case of relatively few individuals who had responded rather poorly to the first dose. Re-immunization after 6 months produced circulating vibriocidal antibody levels approximately the same as those occurring after the initial immunization. These studies suggest that the immunization requirements of children and adults may differ. In very young children, 2 doses of vaccine may be required to induce adequate antibody production. However, adults usually respond well to a single dose of vaccine and only the relatively few "poor responders" are further benefited by a second dose. A single dose of antigen may, therefore, be effective under emergency conditions or in other mass vaccination programmes, but a second dose given 7–10 days later should ensure a good immune response in practically all persons. In children under 5 years of age, the administration of a second dose may be of considerably greater importance than in adults.

Serological methods have also been employed to study the response to different dosages of cholera vaccine. Adult human volunteers in the United States of America were given single doses of 3 vaccines having

anti-Inaba potency equivalent to 0.1, 1.0 and 20 times the mouse-protective potency of the United States Inaba reference vaccine. The response to the vaccine of lowest potency was very much below the response to the vaccine of "standard" potency. However, the vibriocidal titres produced in the group receiving the largest antigen dosage were not significantly higher than those produced by the vaccine of standard potency. Repeated sampling over the ensuing 6 months demonstrated that antibody titres in all groups declined at approximately the same rate. These data suggest that in adults, at least, many of the vaccines in use today are producing antibody responses approaching the maximum, and although less potent vaccine may produce poorer responses, markedly increased antigenic potency will not bring much improvement in immunity.

The short duration of the immunity produced by cholera vaccine is a major problem in the practical control of cholera. With one exception, field studies have demonstrated that immunity following the use of plain vaccines declines substantially after 3–4 months and is undetectable within a year. The exception was the East Pakistan field trial of 1963–1964, where a vaccine of unusually high Inaba mouse-protective potency (but which produced more reaction than other vaccines) provided protection against cholera of the Inaba serotype that persisted for at least 18 months. Efforts to duplicate this effect with other vaccines having acceptable freedom from toxicity have not been successful.

Response to immunization with cholera vaccine is quite rapid. Marked increases in circulating vibriocidal antibody have been demonstrated in children 1 week after their second dose and in adults 1 week after a single dose. In adults, maximum vibriocidal titres against both the Ogawa and Inaba serotypes were obtained approximately 2 weeks after immunization with bivalent vaccines. There are many different types of situation where cholera vaccine is used to produce immunity. It is likely that no single immunization procedure or schedule will be applicable in all instances.

ANTITOXIC IMMUNITY

A variety of toxic substances are produced by the cholera vibrio in cultures and even in the stools of cholera patients. This has led to confusion in the use of the term "cholera toxin", and Burrows has proposed that the various toxic substances be separated into three general types, based upon heat stability and dialysability. The situation is now somewhat clearer and there is general agreement that the toxic principle primarily responsible for fluid accumulation in the gut is a heat-labile, non-dialysable antigenic protein that appears to act upon the intestinal

mucosa in such a way as to grossly stimulate the active secretion of fluid and electrolytes into the lumen. When injected into rabbit or guinea-pig skin this substance, or an extremely closely related substance, can increase the permeability of skin capillaries, resulting in local oedema and in leakage of blue-dyed albumin in animals that have received intravenous injections of pontamine blue.

Although there is not complete agreement that the fluid accumulation activity and the skin permeability activity are due to precisely the same molecular moiety, both types of action have been demonstrated in purified preparations that appear to be molecularly homogeneous by several analytical methods, and both activities are neutralized by antitoxins that appear to have a single specificity. For the purposes of this discussion, these activities will be considered manifestations of the same entity, which will be called "cholera toxin". Unfortunately, general agreement has not yet been reached upon a single name for cholera toxin, and various designations appear in current literature such as enterotoxin, exoenterotoxin, ileal loop toxin, cholera exotoxin, fluid accumulation factor, permeability factor, and choleragen. However, it is important to exclude the heat-stabile non-dialysable "endotoxic" substances that would be included in Type 1 in the Burrows classification, since these are now recognized to be chiefly cell-wall derivates related to the lipo-polysaccharide-containing antigens that are responsible for the antibacterial type of immunity displayed by classical vaccines and purified cell-wall antigens. It is likewise important to distinguish cholera toxin from the heat-stable, dialysable substance capable of inhibiting active sodium transport in the isolated frog skin. This substance's inhibition of sodium and water re-absorption in the intestinal tract was at one time postulated to be the mechanism of cholera diarrhoea. It would be Type 3 toxin in the Burrows classification, but has become of lesser interest since it became apparent that re-absorption is not seriously impaired in cholera. The substance causing the movement of fluid and electrolytes *into* the intestinal tract—the " cholera toxin " with which we are concerned here—would be classified as a Burrows Type 2 toxin.

Investigators have used many different techniques for assaying both toxin and antitoxin, and at least two different antitoxin units have been proposed. In this discussion, therefore, the mention of specific amounts of toxin and antitoxin is avoided.

Up to now no information is available concerning the ability of antitoxic immunity to prevent the occurrence of clinical cholera in man. Circulating antitoxin is demonstrable in most persons 1 week after the onset of cholera, and human volunteers who have been injected with 1 dose of semi-purified cholera toxoid, followed by 2 doses mixed with aluminium phosphate, have produced antitoxin.

Most available information about antitoxic immunity has been derived from animal experiments. Active immunization protects dogs from the effects of intestinally administered cholera toxin. Experiments in which the circulation of immunized dogs has been connected to the blood supply of intestinal segments of non-immunized dogs, and *vice versa*, have demonstrated that intestinal immunity to cholera toxin is provided by circulating antitoxin rather than by local tissue immunity. Dogs immunized with purified toxin in Freund adjuvant have been found to be protected from orogastric doses of live vibrio that produce extensive diarrhoea or death in unimmunized animals. Protection was still strong 10 months after immunization.

Although cholera toxin appears to produce no systemic toxicity in the amounts that may be absorbed in the natural infection, its parenteral injection can provoke considerable local oedema, and mice are killed by the intravenous injection of relatively small doses. Consequently, conversion to a toxoid will probably be necessary in order to permit the injection of sufficient antigen to produce good antitoxin responses but no undesirable local or systemic reaction. The toxoiding of both crude and highly purified cholera toxin has recently been accomplished, and the antigenicity of the toxoids in animals appears to be somewhat superior to that of the toxins from which they were derived.

Experiments in animals indicate that the antitoxin response from a single dose of cholera toxin or toxoid is relatively poor and irregular, but that a second dose given 2 or more weeks later produces a booster-type response. The rate of decline of circulating antitoxin is of considerable importance. Limited experiments in rabbits suggest that antitoxin levels fall to about one-tenth of their original peak values over a 12-month period.

Since cholera is now recognized to be an intraluminal intestinal infection that produces its clinical effects by the elaboration of a toxin, the concept of protection through the creation of antitoxic immunity is appealingly logical, and animal experimentation has encouraged the belief that it may be feasible.

RELEVANT LITERATURE

Azurin, J. C. et al. (1967) A controlled field trial of the effectiveness of cholera and cholera El Tor vaccines in the Philippines, *Bull. Wld Hlth Org.*, **37**, 703–727

Benenson, A. S., Saad, A., Mosley, W. H. & Ahmed, A. (1968) Serological studies in cholera. 3. Serum toxin neutralization—rise in titre in response to infection with *Vibrio cholerae*, and the level in the "normal" population of East Pakistan, *Bull. Wld Hlth Org.*, **38**, 287–295

Burrows, W. (1968) Cholera toxins, *Ann. Rev. Microbiol.*, **22**, 245–268

Das Gupta, A. et al. (1967) Controlled field trial of the effectiveness of cholera and cholera El Tor vaccines in Calcutta, *Bull. Wld Hlth Org.*, **37**, 371–385

Feeley, J. C. & Pittman, M. (1962) *Report, Meeting of Laboratory Workers on Laboratory and Field Studies of Cholera Vaccines* (Document WHO/BD/Ch./2), Annex III, pp. 1–5

Feeley, J. C. & Pittman, M. (1965) Laboratory assays of cholera vaccine used in field trial in East Pakistan, *Lancet*, **1**, 449–450

Feeley, J. C. & Roberts, C. O. (1969) Immunological responses of laboratory animals to cholera vaccines, toxin, and toxoid, *Tex. Rep. Biol. Med.*, **27** (Suppl. 1), 213–226

Finkelstein, R. A. (1969) The role of choleragen in the pathogenesis and immunology of cholera, *Tex. Rep. Biol. Med.*, **27** (Suppl. 1), 181–201

Finkelstein, R. A. & Peterson, J. W. (1970) *In vitro* detection of antibody to cholera enterotoxin in cholera patients and laboratory animals, *Inf. Immun.*, **1**, 21–29

Freter, R. (1969) Studies of the mechanism of action of intestinal antibody in experimental cholera, *Tex. Rep. Biol. Med.*, **27** (Suppl. 1), 299–316

Greenough, W. B. III & Carpenter, C. C. J. (1969) Fluid loss in cholera—a current perspective, *Tex. Rep. Biol. Med.*, **27** (Suppl. 1), 203–212

McCormack, W. M. et al. (1969) Report of the 1966–67 cholera vaccine field trial in rural East Pakistan. 3. The lack of effect of prior vaccination or circulating vibriocidal antibody on the severity of clini al cholera, *Bull. Wld Hlth Org.*, **40**, 199–204

Mosley, W. H. (1969) The role of immunity in cholera: a review of epidemiological and serological studies, *Tex. Rep. Biol. Med.*, **27** (Suppl. 1), 227–241

Mosley, W. H., Benenson, A. S. & Barui, R. (1968) A serological survey for cholera antibodies in rural East Pakistan. 1. The distribution of antibody in the control population of a cholera-vaccine field-trial area and the relation of antibody titre to the pattern of endemic cholera. 2. A comparison of antibody titres in the immunized and control populations of a cholera-vaccine field-trial area and the relation of antibody titre to cholera case rate, *Bull. Wld Hlth Org.*, **38**, 327–346

Mosley, W. H., Ahmad, S., Benenson, A. S. & Ahmed, A. (1968) The relationship of vibriocidal antibody titre to susceptibility to cholera in family contacts of cholera patients, *Bull. Wld Hlth Org.*, **38**, 777–785

Mosley, W. H. et al. (1969) Report of the 1966–67 cholera vaccine field trial in rural East Pakistan. 1. Study design and results of the first year of observation. 2. Results of the serological surveys in the study population—the relationship of case rate to antibody titre and an estimate of the inapparent infection rate with *Vibrio cholerae*, *Bull. Wld Hlth Org.*, **40**, 177–197

Mosley, W. H. et al. (1970) The 1968–69 cholera vaccine field trial in rural East Pakistan: effectiveness of monovalent Ogawa and Inaba vaccines and a purified Inaba antigen, with comparative results of serological and animal protection tests, *J. infect. Dis.*, **121** (Suppl.), S1-S9

Northrup, R. S. et al. (1970) Immunoglobulins and antibody activity in the intestine and serum in cholera, *J. infect. Dis.*, **121** (Suppl.), S137-S146

Philippines Cholera Committee (1968) A controlled field trial of the effectiveness of various doses of cholera El Tor vaccine in the Philippines, *Bull. Wld Hlth Org.*, **38**, 917–923

Sack, R. B. & Carpenter, C. C. J. (1969) Experimental canine cholera. I. Development of the model. II. Production by cell-free culture filtrates of *Vibrio cholerae*. III. Serologic studies and re-challenge experiments, *J. infect. Dis.*, **119**, 138–164

Verwey, W. F. et al. (1969) Serological responses of human volunteers to cholera vaccine, *Tex. Rep. Biol. Med.*, **27** (Suppl. 1), 244–274

Watanabe, Y. et al. (1969) Some of the properties of mouse protective antigens derived from *Vibrio cholerae*, *Tex Rep. Biol. Med.*, **27** (Suppl. 1), 275–298

CHAPTER 13

CHOLERA VACCINES

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The cholera vaccine employed at present in most areas of the world consists of a saline suspension of approximately 8×10^9 killed cells of *Vibrio cholerae* per ml, composed of equal numbers of the Ogawa and Inaba serotypes. It was not until the 1960's that properly controlled field trials were conducted in Calcutta (Das Gupta et al., 1967), the Philippines (Azurin et al., 1967; Philippines Cholera Committee, 1968), and in Dacca (Oseasohn et al., 1965; Benenson et al., 1968; Mosley et al., 1969; Mosley et al., 1970).

A variety of vaccines, some experimental, have been employed in these field trials, and their apparent efficacy has varied widely, as have the results from one trial to another. In general terms, maximum protection in the range of 30–90% has been achieved, and at least some significant protection has been observed to extend over a period of 3–6 months. Longer protection has been observed with an experimental oil-adjuvant vaccine (Azurin et al., 1967) and with a fluid vaccine of high mouse potency (Benenson et al., 1968). There are some suggestions that longer protection is achieved in adults in highly endemic areas (Benenson et al., 1968).

Thus, it would appear that the vaccines now available do indeed give some significant protection, although it is of rather limited duration; this is ample to justify their continued use in the face of a significant epidemic threat, as a means of reducing the probable number of acute cases of cholera. Vaccine should be used in conjunction with other public health measures. In spite of the requirement for an International Certificate of Vaccination for travel from infected areas, there is little evidence that the spread of the disease from one country to another has been prevented.

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PRODUCTION AND TESTING OF CHOLERA VACCINE

Detailed requirements for cholera vaccine have been published by WHO.[1] Cholera vaccine should be prepared in accordance with these requirements and those of the national control authority of the country making the vaccine. Only vaccines that meet these requirements may be used for vaccinations to be recorded on the International Certificate of Vaccination or Revaccination against Cholera. The requirements are intended to ensure, as far as possible, that vaccines produced in accordance with their provisions will be as effective as those used in controlled field trials conducted to date. Some of the major problems involved in the production and testing of cholera vaccine are discussed briefly in the following sections.

Vaccine production strains

It is imperative that only smooth, authentic cultures of Ogawa and Inaba serotypes of *V. cholerae* be employed. Whether so-called El Tor strains should be used is controversial. The differentiating features (e.g., phage or polymyxin B sensitivity, haemolysin production, chicken cell agglutination, and Voges-Proskauer reactions) do not seem to be connected with antigenic structure. In their major antigenic features, El Tor and classical strains of *V. cholerae* are identical, and both can be typed as Ogawa or Inaba serotypes. In mouse-protection tests (Pittman & Feeley, 1963), vaccines made from classical strains were equally effective against either El Tor or classical challenge. In field trials, both classical and El Tor vaccines have shown protection against El Tor cholera (Azurin et al., 1967). Fractionated antigens from El Tor strains (Watanabe & Verwey, 1965; Verwey et al., 1965) have given protection against classical cholera (Benenson et al., 1968; Mosley et al., 1970).

For the time being, it is essential to continue the practice of using both serotypes in the vaccine. Field trials in East Pakistan have shown that monovalent Ogawa whole-cell vaccine gives no protection against Inaba cholera, while Inaba vaccine is highly protective (Mosley et al., 1970). The degree of cross-protection conferred by either Ogawa or Inaba vaccine against Ogawa cholera has not yet been demonstrated in field trials. Whatever strains are used, it is essential that they be preserved by a system (e.g., freeze-drying) that will minimize cultural and antigenic dissociation, which occurs rapidly with *V. cholerae*.

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[1] Requirements for cholera vaccine (Requirements for biological substances No. 4), revised 1968. In: WHO Expert Committee on Biological Standardization (1969) *Twenty-first report* (*Wld Hlth Org. techn. Rep. Ser.*, No. 413), Annex 1, pp. 27-44.

Repeated serial transfers of cultures over a long period of time should be avoided in the maintenance of seed cultures.

Media for vaccine production

Either solid or liquid media may be used for vaccine production, but in either case the culture medium should not contain blood group substances or other materials that are antigenic and capable of inducing hypersensitivity in man. Highly hydrolysed casein hydrolysate media are very satisfactory for this purpose but a variety of peptone or meat infusion media may be employed. It appears that solid media are preferred in most laboratories, since standardization of the bacterial content of harvested organisms is somewhat easier and there is less likelihood of contaminating the vaccine with ingredients derived from the media. Some laboratories that use liquid media containing highly hydrolysed casein hydrolysates follow the practice of simply diluting the whole culture to the proper bacterial concentration, in which case the vaccine contains all the ingredients of the medium. If this is done, it is essential to ensure that the medium is nonallergenic. In general, the period of growth should not exceed 24 hours at 35–37°C, since autolysis of the organisms will interfere with proper determination of bacterial content.

Standardization of the bacterial harvest

The bacterial content of the harvested suspension should be determined within 2 hours (never after more than 6 hours) and always before the addition of any killing agent such as phenol, which causes marked autolysis. The bacterial content of the final vaccine (8×10^9 organisms/ml, composed of equal numbers of the Ogawa and Inaba serotypes) should be based on this early determination on the harvested suspensions, since—owing to autolysis—later determinations will not reveal the true count. The bacterial content is most frequently determined by a turbidimetric method standardized by the International Reference Preparation for Opacity or an equivalent turbidity reference preparation. Other methods, such as direct microscopic bacterial counts, nitrogen determinations, and dry weight determinations, may be useful under defined circumstances, but the turbidimetric procedure is much more practical and convenient.

Killing agents and preservatives

The most commonly used killing and preserving agent is 0.5% phenol, although 0.1–0.3% formalin (37% formaldehyde solution), mild heat (56°C for 30 min), and mercurials (e.g., 0.01% thiomersal) have also been employed. There is no sound evidence from vaccine use in man

of the advantage of any particular preservative, although laboratory experiments (Feeley, unpublished data) have shown that mild heat (56°C for 30 mn) was rather deleterious to potency. No differences were observed among other killing agents, but it is of some interest that all killing methods resulted in a product that was less potent than a living vaccine. If formalin is used as a killing agent, it must be removed or neutralized with sodium bisulfite to prevent painful reactions in recipients; some other preservative must then be employed in the final product.

It should be pointed out that some preservatives, especially phenol, cause marked lysis of the bacterial suspension, which may therefore have very little turbidity. There is no evidence that this is deleterious, but the observation has been a cause of some concern to physicians administering the vaccine, who thought it to be "weak" because of the relative lack of turbidity.

Potency tests

Cholera vaccines are frequently tested for antigenic potency by a mouse-protection test. Separate groups of mice are immunized intraperitoneally or subcutaneously with graded doses of the vaccine under test, and with graded doses of the International Reference Preparations of Cholera Vaccine (or equivalent national reference preparations). Separate tests are conducted for Ogawa and Inaba serotypes. Seven to 14 days after immunization, the mice are challenged intraperitoneally with Ogawa and Inaba strains suspended in gastric mucin to enhance virulence. The median effective dose (ED_{50}) of the test vaccine and the reference vaccine is calculated. The vaccine should confer the same degree of protection as the reference preparations against both serotypes.

In spite of the fact that the disease in the mouse is a fulminating septicaemia bearing essentially no relationship to cholera in man, potency as determined by this test has correlated quite well with the apparent efficacy of vaccines as demonstrated by controlled field trials in man.

Since many laboratories are not equipped to perform mouse-protection tests, the vaccine may also be tested by comparing its ability to induce the production of antibody (e.g., agglutinating or vibriocidal antibody) with that of the reference vaccines. Guinea-pigs, rabbits, or mice may be used for this test.

Other tests

Each lot of vaccine should pass appropriate sterility and innocuity or safety tests. Safety tests are performed by injecting the vaccine into mice and guinea-pigs. A variety of other tests, such as nitrogen content,

preservative content and pH, are advisable as quality control checks on the final product. In addition, newly developed vaccines and certain batches of routine production vaccines should be carefully studied in human subjects to ensure that they give no unusual or untoward reactions.

ADMINISTRATION AND USE

Dosage

Cholera vaccine is usually given subcutaneously or intramuscularly in two doses of 0.5 ml and 1.0 ml, 7–28 days apart. For mass immunization, jet injector devices are very useful. Children under the age of 10 years are often given reduced doses (0.1–0.3 ml), although children seem to tolerate adult doses well.

When mass immunization is being carried out under epidemic conditions, it is often not possible to give two doses; in this case, a single dose of 1.0 ml is preferred. It is comforting to note that most of the field trial experience has been based on a single dose. In one field trial, somewhat higher protection in children was obtained with 2 doses (Mosley et al., 1969), although in another trial one dose was found to be just as effective as two (Philippines Cholera Committee, 1968).

Booster doses are generally given every 6 months, and this seems advisable in view of the apparently short period of immunity induced by most vaccines. However, there are no adequate human data to show the protective effects of the booster dose given at 6 months.

Reactions

Immunization is generally accompanied by mild to moderate tenderness at the injection site, although slightly more severe local reactions occur in some individuals. These usually persist for 2–3 days. The local reaction may be accompanied by mild fever, malaise, and headache. These symptoms may be ameliorated by administration of salicylates. More serious reactions are most frequently the result of an excessive antigen content in the vaccine (i.e., significantly more than 8000 million organisms/ml).

FUTURE OUTLOOK

A great deal of research is now in progress in many laboratories throughout the world to develop more effective immunization against cholera. The more promising lines of investigation are listed below:

1. *Toxoid vaccines*

It is now quite clear that the severe fluid and electrolyte loss in cholera is actually caused by a heat-labile antigenic exotoxin elaborated by *Vibrio cholerae* while multiplying in the gut (Burrows, 1968). This toxin has now been prepared in highly purified form by several investigators (Richardson & Evans, 1968; Finkelstein & Lo Spalluto, 1969; Kaur et al., 1969). The antigenic activity of the toxin is not represented in most currently available cholera vaccines (Feeley & Roberts, 1969; Mosley & Ahmed, 1969). Research efforts are under way to prepare suitable toxoids for field trial in man to test the hypothesis that antitoxic immunity may be effective in preventing this disease.

2. *Purified antigens*

Purified immunogenic fractions have been developed and have the theoretical advantage of eliminating non-essential and reaction-causing substances from the vaccine. A purified Ogawa lipopolysaccharide (Watanabe & Verwey, 1965) has given limited protection against Inaba cholera (Benenson et al., 1968), but it has not been evaluated against Ogawa cholera. A purified Inaba fraction (Verwey et al., 1965) has shown excellent protection against Inaba cholera (Mosley et al., 1970).

3. *Adjuvants*

An oil-adjuvant cholera vaccine (Ogonuki et al., 1967) has given impressive long-term protection in the Philippines (Azurin et al., 1967), but unfortunately produces too strong a local reaction for routine use. Other adjuvants (e.g., aluminium hydroxide) offer considerable potential for increasing the level and duration of immunity (Joó et al., 1967).

4. *Oral vaccine*

It has been suggested that oral vaccine is more effective than parenteral vaccine in stimulating a local immune response (production of copro-antibody) in the gut (Freter, 1962; Freter & Gangarosa, 1963). Present information suggests that large and frequent doses of killed oral vaccines would be needed, but some efforts are being directed towards the development of living avirulent or attenuated living vaccines (Mukerjee, 1963; Sanyal & Mukerjee, 1969).

REFERENCES

Azurin, J. C. et al. (1967) *Bull. Wld Hlth Org.*, **37**, 703
Benenson, A. S. et al. (1968) *Bull. Wld Hlth Org.*, **38**, 359
Burrows, W. (1968) *Ann. Rev. Microbiol.*, **22**, 245

Das Gupta, A. et al. (1967) *Bull. Wld Hlth Org.*, **37**, 371
Feeley, J. C. & Robert, C. O. (1969) *Tex. Rep. Biol. Med.*, **27**, suppl. 1, 213
Finkelstein, R. A. & Lo Spalluto, J. J. (1969) *J. exp. Med.*, **130**, 185
Freter, R. (1962) *J. infect. Dis.*, **111**, 37
Freter, R. & Gangarosa, E. J. (1963) *J. Immunol.*, **91**, 724
Joó, I. et al. (1967) *Z. Immun.-Forsch.*, **133**, 317
Kaur, J. et al. (1969) *J. Bact.*, **100**, 985
Mosley, W. H. & Ahmed, A. (1969) *J. Bact.*, **100**, 547
Mosley, W. H. et al. (1969) *Bull. Wld Hlth Org.*, **40**, 177
Mosley, W. H. et al. (1970) *J. infect. Dis.*, **121** (suppl.), S1-9
Mukerjee, S. (1963) *Bull. Wld Hlth Org.*, **29**, 753
Ogonuki, H. et al. (1967) *Bull. Wld Hlth Org.*, **37**, 729
Oseasohn, R. O. et al. (1965) *Lancet*, **1**, 450
Philippines Cholera Committee (1968) *Bull. Wld Hlth Org.*, **38**, 917
Pittman, M. & Feeley, J. C. (1963) *Bull. Wld Hlth Org.*, **28**, 379
Richardson, S. H. & Evans, D. J. (1968) *J. Bact.*, **96**, 1443
Sanyal, S. C. & Mukerjee, S. (1969) *Bull. Wld Hlth Org.*, **40**, 503
Verwey, W. F. et al. (1965) In: *Proceedings of the Cholera Research Symposium, Honolulu, 1965*, Washington, D.C., Government Printing Office (PHS Publication No. 1328)
Watanabe, Y. & Verwey, W. F. (1965) *Bull. Wld Hlth Org.*, **32**, 809
WHO Expert Committee on Biological Standardization (1969) *Twenty-first report* (*Wld Hlth Org. techn. Rep. Ser.*, No. 413)

CHAPTER 14

ENVIRONMENTAL HEALTH MEASURES IN CHOLERA CONTROL

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Contaminated water is an important vehicle for the rapid spread of a cholera epidemic; insanitary personal and food habits of the population are largely responsible for the persistence and intensification of transmission when the epidemic appears.

There is no doubt that the first preoccupation of health authorities in countries at risk of a cholera epidemic should be the preservation of the good quality of water delivered by the public supply system, through constant vigilance over the operation and maintenance of all its components, from the water source to the most remote house connected with the distribution network. In fact, the responsibility of the health agency should extend to making safe the water used by that portion of the population not served by the public system when they are threatened by cholera.

The most effective protection consists of eliminating as far as possible all sources of contamination that may endanger the safety of public and private water systems. Human faeces are the main source of contamination and cholera outbreaks are typically associated with situations in which two factors are present: the water supply is unsafe or exposed to a high risk of contamination, and defecation habits and excreta disposal installations are such that they favour, rather than control, the spread of contamination.

WATER SUPPLY

Measures applicable to the water supply of urban centres

Inspection. Officials of the water authority and the health agency should inspect jointly all sources of pollution that may endanger the

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quality of water in its natural state before it reaches the treatment plant or distribution system. Pollution can take place in:

1. Catchment areas that serve as water supply sources. The presence of human dwellings, barns and stables requires careful inspection; sewer and septic tank discharges, defective latrines, and manure piles are the major sources of pollution; indiscriminate defecation and insanitary disposal of excreta may be responsible for widespread pollution. The best method of control is to remove all human and animal habitation from the catchment area and divert polluted discharges downstream, beyond the point of water intake. As this is not always possible, sanitary latrines should be built or the discharges disinfected.

2. Water courses. Streams and channels present difficult problems of inspection and control as they may also be used for irrigation and navigation. People living on the banks will naturally use this water for all their needs, including bathing and drinking, without any precaution. While it is desirable to keep the whole course under control, it is only feasible to maintain intense surveillance within a relatively short distance from the water intake. When there is the possibility of reverse flow, continuous protection should be provided at least 500 m upstream and for an adequate distance downstream. If sampling for water quality has been carried out as a routine, the records will permit the occurrence of fresh pollution to be immediately detected.

3. Wells and springs. When used as sources for public water supplies, wells and springs present a localized problem that is not difficult to solve. All sources of pollution should be removed from an area around the source at least 30 m in radius, unnecessary access should be prevented (e.g., by means of a fence), and surface water should be diverted out of this area.

4. Reservoirs. Sometimes reservoirs are used for recreational purposes, such as boating and fishing; during an emergency or threatened emergency all such use should be prohibited. People should be informed of this prohibition and surveillance instituted to ensure that it is observed.

Water works operation. The operators or superintendents of waterworks should ensure that every component of the system functions at maximum efficiency. Sanitary engineers of the health administration should ascertain that the works supplying large towns are adequately supervised. Special attention should be paid to the efficiency of water treatment processes. Lack of trained operators can be remedied by arranging short crash courses for water plant operators; WHO or bilateral assistance is available on request.

During an emergency, the chlorine dosage should be increased so as to give a higher degree of protection; this may imply additional equipment that should be available before the epidemic strikes. In smaller towns, chlorinators of the solution-feed type could be fabricated locally. Ample stocks of chlorine should be procured in advance for use during emergencies. Proper sanitary facilities for the staff should be provided in all water treatment plants so as to permit a high degree of personal cleanliness.

Distribution system. As no water distribution system is leak-proof, the risk of contamination is always present. This risk becomes a certainty where the supply is intermittent or when the system is worked at low pressure, a situation that exists in most towns of developing countries.

1. *Disinfection.* Chlorine, either free or as chlorine dioxide, is the most effective water disinfectant; a residual of 0.2–0.4 mg/litre at the waterworks, which is the normal practice, will not give much protection when the system operates intermittently or at low pressure. This dosage must be increased during emergencies, and chlorination applied not only at the waterworks but also at strategic points in the distribution system. Organic matter adhering to the pipes will produce odour and other problems when the chlorine dosage is increased; this effect may last for some weeks, depending on the amount of organic matter in the pipes. Notwithstanding the possibility of complaints about odour and taste in the water, the increased dosage of chlorine is strongly recommended as it is the most effective means of protecting water in a period of threatened or active epidemics.

Emergency chlorination of the distribution system could be applied at booster pumping stations or other convenient points. Equipment delivering chlorine gas is preferable for emergency application because of its reliability and easy operation. When this is not possible, the alternative is the use of solution-feed chlorinators; water authorities should find no difficulty whatever in installing such chlorinators and preparing the chlorine solution, as instructions are readily available in many publications (see, for example, the WHO monographs No. 42 and No. 49 in the list of publications on p. 109).

The effectiveness of chlorination depends on the availability of a residual of free chlorine in the water. Combined chlorine is less effective, and its disinfecting power decreases as the pH value rises to 7.5 or more.

2. *Detection and repair of leaks.* All distribution systems have their weak points, which are liable to leaks and breakage because of the age and deterioration of pipes, joints and valves, chemicals in the soil,

unstable support, insufficient protection against heavy loads and road traffic, etc. These weak points should be well-known to the network inspectors; other leaks may pass unnoticed and tests on water pressures and flow discharges are required for their detection. Inspection and detection work should be intensified and immediate repairs should be performed when there is a threat or an outbreak of an epidemic.

3. *Sampling.* Water sampling for chlorine residual determination should be taken from points farthest away from the application points to ascertain the efficacy of chlorination. Samples for bacteriological examination should be taken particularly from the crowded slum areas where sanitation is poor.

Fringe or slum areas. Slums are particularly susceptible to epidemics as they are never served adequately by utility services. Control measures, therefore, should concentrate first of all on these areas. Their sources of water supply may be open wells, springs and streams, which are always liable to pollution. A comprehensive disinfection programme must be initiated and all feasible measures for the sanitary protection of these sources must be taken without delay.

In addition, the distribution system should be extended to these areas whenever possible by the temporary installation of galvanized iron or plastic pipes and a number of street stand posts. A second alternative would be the provision of water through a tanker system. It is essential to ensure that tankers are adequately sterilized and additional chlorine is applied to protect the water against the risks of careless handling.

Public places. Water installations at schools, hospitals, railway stations, and other public places must be very carefully examined and any sanitary deficiency corrected, as they are particularly vulnerable to pollution when unsanitary conditions prevail.

It is not unusual to find leaking valves, stand posts without faucets, valve chambers below ground level subject to surface contamination, and a host of other sanitary deficiencies; these should be rectified.

Public fountains should be made or modified so as to prevent the direct contact of the user's mouth with the faucet or its contamination in any other way.

The storage of water for drinking is widely practised when the supply is inadequate and infrequent, or when the water requires cooling in earthenware pots. This practice involves many risks as contamination of these containers occurs unless proper precautions are taken. The containers should therefore be washed frequently and thoroughly, adding a weak disinfectant if possible; they should be filled directly from a faucet of safe water, and provided with a small tap at the bottom. A

hole can be drilled for this purpose, or potters may be asked to provide already bored containers. In schools, where many of these pots may be used, they should be filled directly without any handling and always kept covered, and the service tap at the bottom should be the only part accessible to the users.

Ice and bottled waters. The preparation of ice and bottled waters requires the same precautions as are applicable to drinking water. The source of supply at all factories should be periodically inspected, and samples for bacteriological examination should be taken frequently. The sanitary conditions of the premises and the bottle-washing process deserve special attention during routine and surprise visits by public health inspectors. Manufacturers should be made responsible for the quality of the water used, through additional disinfection where necessary.

Bacteriological examination of water. All waterworks in large towns should be provided with laboratory facilities for checking the bacteriological quality of the water supplied to the public. This control should be intensified when there is a threat or actual occurrence of an epidemic. Whenever an indication of contamination becomes apparent, the first step should be to increase the dosage of chlorine and warn the consumers to take the necessary precautions. The point of contamination should be immediately located and remedial action taken without delay.

The recommendation that drinking water should be boiled is least likely to be followed by the people most in need; it involves fuel, utensils, and time for preparation and cooling. The warning should nevertheless be given to the public; they should also be encouraged to drink hot beverages, tea, coffee, etc. instead of suspected water.

The membrane filtration method for bacteriological examination, total bacteria count and coliform count, has proved its practical value in outlying areas. Portable kits are available and not very expensive. The water sample is passed through a sterile membrane filter that is placed in a plastic Petri dish for incubation. Portable incubators are also available, but in their absence the Petri dish can be wrapped in polyethylene foil and placed close to the body for incubation. For a large number of samples the portable incubator is indispensable. For coliform count, double-field monitors with endo-medium should be ordered; they are very light and instructions for their use are attached to each carton. The usable period of these monitors is about 9 months; they should therefore be ordered in relatively small quantities and preferably shipped by air freight. In emergencies, when time is an important

factor, bacterial contamination can be detected after 8–12 hours of incubation; final results indicating that contamination is of faecal origin require 18–24 hours. It is course, strongly recommended that the test should be carried to completion.

Measures applicable to the water supply of rural communities

Dug wells. The work required for the sanitary protection of dug wells is time-consuming and, as there are so many privately owned wells, it is not feasible to undertake a comprehensive programme of improvement during the short period before an epidemic occurs or while one is in progress. However, large wells serving whole villages may be protected by village councils or other local authorities during the course of a mass cholera campaign.

Various methods for the sanitary protection of dug wells are shown in the WHO Monograph No. 42 (see p. 109).

During an emergency, the disinfection of dug wells may be the most practical way of protecting these water sources. Several simple and effective methods, suitable for use in emergency conditions, have been devised for the application of a constant dosage of chlorine to well water.

The pot method of chlorination has been used with success in various countries; the double pot method is an improvement devised by the Central Public Health Engineering Research Institute, Nagpur, India. This method uses two cylindrical pots, one placed inside the other. The inside height and diameter are 30 cm and 25 cm, respectively, for the outer pot, and 28 cm and 16 cm, respectively, for the inner pot. A hole 1 cm in diameter is made in each pot; in the inner pot the hole is in the upper portion, near the rim, and in the outer pot it is 4 cm above the bottom.

A mixture of 1 kg of bleaching powder and 2 kg of coarse sand (approx. 2 mm in diameter) is prepared and slightly moistened with water. The inner pot is filled with this mixture up to 3 cm below the level of the hole. The inner pot is introduced into the outer one, and the mouth of the latter closed with polyethylene foil. The use of two pots makes it is possible to have larger holes without the risk of over-chlorination.

The double pot is lowered into the well by means of a rope attached to the well kerb. The pot should be immersed at least 1 m below the water level to prevent damage by the buckets used for drawing water. It has been found that this device works satisfactorily for 2–3 weeks in small household wells containing about 4500 litres of water and having a draw-off rate of 360–450 litres per day.

Drilled wells. For this type of water supply, the only emergency action required is to inspect the pump installation to ensure that no surface contamination enters the well. Particular attention should be paid to the seal between the concrete platform and the well casing, and to the gasket in the joint between the drop pipe and the base of the pump. It is also important to ascertain that water is drained away.

Springs. The structure that protects the spring from contamination by surface drainage and other pollution should be carefully inspected for fissures and other damage, and immediately repaired if necessary.

Ponds, lakes, streams, and other surface sources. These are the most difficult sources to keep under sanitary conditions, and should only be used as a last resort. Where possible, arrangements for extracting water through a sand filter should be made. A number of ideas are given in the document *The village tank as a source of drinking water* (WHO/CWS/RD/69.1), which is available from WHO on request.

In an emergency, efforts should be made to treat at least any surface water used for drinking and cooking. Village councils could post guards to protect the source from pollution; strict vigilance should be directed to the places most frequented by villagers.

Portable (package) water-treatment plants

Truck-mounted portable water-treatment plants are extremely useful in emergencies. They provide means for filtering, disinfection, and chemical treatment in one self-contained unit. They are used in particular by the military or civil defence organizations.

The characteristics of the truck and motor must be carefully studied, keeping in mind the terrain where they will be expected to operate. These plants are expensive and can prove very wasteful if no provision is made for sufficient spares and trained operators. They are no substitute for the conventional type of permanent treatment plant, on either a cost or efficiency basis. They are, however, very valuable in emergencies and catastrophies if properly maintained and kept in readiness. It is wise to provide mobile plants in areas repeatedly exposed to natural disasters and epidemics, so as to ensure a safe supply of water until more permanent relief measures can be taken.

Truck or trailer tankers supplied with pumps may also prove invaluable during emergencies. These tankers should be kept in readiness and not used as a permanent means of supply, for if they are it will not be possible to withdraw them from the areas they normally serve when an emergency occurs.

Small-scale purification of water

Information on boiling, chemical disinfection, filtration and storage of water, suitable for application in the household, has been given by Clark.[1]

SEWERAGE

Maintenance and operation of sewerage systems

In cities, the unavoidable proximity of sewers and water supply pipes constitutes a potential hazard through the infiltration of sewage into the water system. Sewers should be inspected periodically for leaking joints and cracks; this necessitates uncovering the sewer line and effecting any repairs or replacements that may be required. Because of the cost and the inconvenience involved, such inspections cannot be carried out frequently enough to ensure that no leakage takes place, and the only feasible means of preventing contamination of the water system is to maintain at all times a high water pressure in the supply lines. When there is a risk of a cholera outbreak and during an epidemic, the water service must not be interrupted nor the pressure in the pipe network lowered at any time, so as to prevent negative pressures which cause water surrounding the pipes to be sucked in through defective joints and fissures.

Outfall sewer. The most dangerous point in the sewerage system is the site where the outfall sewer discharges into the receiving body of water, canal, river, lake, sea, etc. The water at this point is highly polluted and, during an outbreak of cholera, it will be contaminated by the vibrio. This contamination may spread far and wide and, as there is a constant supply of contaminated matter, it may remain in the receiving stream or reservoir for an indefinite period. The greatest risk arises when these waters are taken out of rivers and canals for irrigating orchards, washing, bathing, and even drinking; lake and sea shores are likewise dangerous to people who frequent them.

Chlorination. The only practical means of controlling this contamination is to chlorinate the sewage, preferably at the treatment plant, if it exists, or at the head of the outfall sewer; as far as practicable, this should be done from the discharge point, so as to allow good mixing and sufficient time for the contact of the disinfectant with the raw

[1] *Bull. Wld Hlth Org.*, 1956, **14**, 820.

sewage. The application of chlorine compounds should be made by mechanical feeders, but in their absence less elaborate devices may be improvised provided the proper dosage is ensured. The amount of chemical should be such as to produce a constant chlorine residual of 0.2–0.5 mg/litre at the outlet. The dosage needed for obtaining this residual varies with the strength, freshness, and other characteristics of the sewage; it should be determined in the laboratory whenever possible, otherwise it can be found by trial in the field until the desired residual is produced. The total dosage of chlorine ordinarily ranges from 15 to 25 mg/litre.

Vigilance. In areas at risk of, or already affected by, a cholera epidemic, strict police action must be enforced to prevent the use of contaminated waters or contact with them. Beaches in the vicinity of the outfall sewer should be closed to the public; irrigation of orchards with polluted water, particularly for growing green-leafed vegetables (lettuce, cabbage, celery, etc.) and soft-skinned bulbs (onions, carrots, radishes, etc.) which are eaten raw, must be prevented.

EXCRETA DISPOSAL

Transmission risks. A large portion of the population living in rural communities and on the fringes of urban areas in developing countries have no means for the disposal of excreta; at best, only few installations are available and these do not offer any guarantee of sanitary protection. Careless habits of defecation and inadequate sanitary facilities are the cause of widespread pollution; the faecal matter is readily transported by rainwater, animal or human feet, and flies or other pests, with the risk of contaminating not only ground water through seepage, but also food, surface water and kitchen and other utensils.

Night soil. In times of cholera epidemics, the bucket system for the collection and disposal of night soil presents a great risk for the handlers of the buckets, containers and carts used for this purpose, as spillage and leakage are hard to prevent. It is also a hazard to the public in general, as careless handling during transport provides many opportunities for scattering part of the contents on the road; people living close to the disposal site are particularly at risk because of the breeding of flies and other vermin. The night soil system can be tolerated under normal conditions if properly designed and operated, if the

cleanliness and disinfection of containers is ensured, and if there is a public sewer for the disposal of the night soil. When there is a risk of a cholera epidemic, this system must be discontinued and replaced by the use of latrines.

Location of latrines. Temporary public latrines should be built before the cholera epidemic strikes, and people without adequate facilities in their homes must be persuaded and even compelled to use them. Careful attention should be paid to their location and construction, so as to prevent any possibility of contaminating water sources. They should be at a lower level than the nearest well, spring or reservoir, but the bottom of the latrine must be at least 1.5 m above the groundwater table. The distance between the latrine and any water source or conduit should be as large as practicable but never less than 30 m; additional precautions should be taken when the soil is fissured (rock and limestone formations).

The trench latrine. The most convenient type of communal latrine for temporary use is the trench latrine that serves a series of seats or squatting holes. The depth of the trench should be between 1 and 2 m, and its width as narrow as can be dug with pick and shovel (40–80 cm); the length should be calculated at the rate of 3–3.5 m (5–6 seats or squatting holes) for every 100 users. A trench of these dimensions may be used for 3–6 weeks. When it has been filled up to 30 cm below the surface level, it must be covered with earth, heaped above ground, and compacted; a new trench must then be dug. It is preferable to build latrine blocks in pairs for the separate use of men and women. Concrete slabs and seats are advisable as they are easier to keep clean, but for expediency and economy's sake they can be replaced by boards set lengthwise to prevent the crumbling of the edges of the trench and crosswise to provide a firm footing. A fence or wall made of matting, canvas, wood, or sheet metal will give the necessary privacy.

It is extremely important to keep the latrine block and its immediate surroundings clean at all times and to disinfect the ground, walls, slabs or boards daily, preferably with a heavy spray of strong chlorine solution, 10–20 mg/litre. The contents of the trench should also be sprayed, or sprinkled with sufficient chlorinated lime in powder form to cover them completely. Chlorine-liberating compounds are the most common disinfectants; they are highly effective, cheap, readily available, and easily handled and applied, but above all they are of universal use, as water, food, sewage, wastes, utensils, clothing, etc. can be made safe without causing damage. Other disinfecting products, such as those based on cresol and phenol, have not the same range of applicability;

they impair the quality of food and other materials and can therefore be used safely only for disinfecting excreta and other wastes, utensils, walls, floors, etc. Iodine has proved to be a good disinfectant; as it does not produce odour or taste it is particularly suitable for disinfecting water and food; a dosage of 5 to 10 mg/litre is effective against bacteria and viruses within 20 minutes.

Public places. Special attention must be given to providing the best possible means of excreta disposal (water closet, chemical toilet, aqua privy, pit, etc.) at those places where cholera transmission is most likely to occur: hospitals, clinics, water treatment plants, pumping stations, markets, restaurants, food stores, etc. The provision of safe water and the proper use and maintenance of these installations are essential if the maximum benefit is to be obtained from these facilities.

FOOD

Transmission risks. The careless and insanitary handling of food carries an important risk of cholera transmission. It is reasonable to assume that food eaten raw or partially cooked is most dangerous; it must be remembered, however, that food is often contaminated after it is cooked. Consequently, the maintenance of sanitary conditions and the observance of correct hygienic practices in the processing, storing, and serving of food in restaurants and other public eating places are of extreme importance, as all previous efforts and precautions to make food fit for human consumption become useless if at the last minute food is exposed to contamination. Experience has proved, however, that the improvement of hygienic standards in eating places is a long process, demanding continuous control through licensing and inspection of premises, penalties for infraction, and the training and health control of food handlers.

Training and inspection. In communities where food control has been inadequate and sanitary measures in eating places have not been enforced, it is impossible to expect a drastic improvement in the situation from one day to the next. However, much can be gained by lecturing food handlers and inspecting premises frequently, by exerting strict supervision of the production, processing, distribution and sale of those food products that are more liable to contamination, and by informing the public about basic precautions for preventing or removing contamination of their food and eating utensils.

Markets and food shops. The control of street food vendors, open markets, and similar places is extremely difficult and special measures are needed to introduce or improve hygienic practice. The provision or control of water supply, sewage disposal, and refuse collection in established markets should be essential items of any campaign directed against cholera. Proper facilities for washing and disinfecting fruits and vegetables should be made available at markets, receiving and distributing centres, and entry control points; as an improvised means of disinfection wire baskets can be provided in which fruits and vegetables are immersed into tanks containing an iodine solution at a strength of 5–10 mg/litre. Existing sewage facilities in markets should be improved and properly maintained; if none are available, sets of trench latrines could offer a suitable solution. Food refuse should be stored in closed containers and removed as frequently as required.

Polluted food. Food contaminated at the source is liable to remain in that state from production to consumption. For this reason, the practice of irrigating vegetable and fruit gardens with sewage-polluted water and of fertilizing the ground with fresh night soil must be prohibited at all times, but particularly when there is a threat of cholera. Fish and shellfish caught in polluted waters for human consumption must be condemned whenever an outbreak of cholera occurs or appears imminent.

FLY CONTROL

Flies and cockroaches have been implicated in the transmission of various alimentary infections, including cholera. Any campaign to prevent or control a cholera outbreak should include drastic measures to combat flies, cockroaches, and other household pests. An intensive campaign of disinfection and disinsection should therefore be instituted.

Disposal of refuse. Refuse is particularly attractive to flies and rodents; its frequent collection and rapid removal away from dwellings should be ensured by employing more personnel and hiring privately owned vehicles if necessary. Conditions in refuse dumps should be carefully inspected and all deficiencies should be corrected, particularly in those located within 3 km of the city limits. If any improvement is impracticable or very expensive, the best solution may be to level and compact the refuse, cover it with earth, and then to abandon the dump and start a new one conveniently located and operated in accordance with accepted sanitary practice. In the worst circumstances, refuse

in open dumps should at least be burnt regularly; the application of crude oil or other fuel may be needed to obtain a more thorough combustion.

Public places. Within the city, markets present a singular situation: because of the production of wastes, the presence of flies, the usually insanitary toilet facilities, and the abundance of unprotected food, transmission risks are high within a very restricted area. Special care should be taken to ensure that all wastes are promptly removed at the end of the day, that food attractive to flies is displayed in fly-proof containers, that food is stored overnight in covered boxes or wire-mesh cages so as to be out of reach of flies and cockroaches, that insect control measures are enforced, and that the premises are always kept clean. As already mentioned, the provision of safe water and proper means of excreta disposal are essential hygienic requirements in markets.

Hospitals, clinics, and other centres of medical treatment are equally important in the transmission of cholera. The presence of suspected or confirmed cases demands the observance of strict sanitary and hygienic measures and scrupulous cleanliness. The improper disposal of wastes, the inadequacy of water installations, and the negligence in kitchens and dining rooms, which not infrequently can be observed in these institutions, must not be tolerated.

Screening. As it is not possible to destroy all flies and cockroaches, or to eliminate their breeding places, an important line of attack is to prevent their access to food and shelter by wire screening of windows and doors, and by other protective measures applicable to dwellings and buildings where food is processed, stored, cooked, sold, or served.

SPECIAL MEASURES OF CHOLERA CONTROL

The previous sections have dealt with control measures that are applicable not only to cholera but also to other communicable diseases where water, wastes, food, and pests intervene in the transmission cycle. The following measures apply especially to cholera.

Handling of victims. The handling and disposal of the dead bodies of cholera victims is of particular importance in the transmission of the disease. Corpses should be considered as foci of infection and treated accordingly; they should be handled only by properly trained and protected personnel, who should wash their hands and other parts of the body that come into contact with the corpses immediately after

each handling, using disinfecting soap; they should change their work garments as frequently as needed to keep them constantly clean. Corpses should be washed with a disinfecting solution and wrapped in shrouds that have been soaked in an antiseptic preparation; chlorine, phenol, and cresol compounds are indicated. All the body orifices should be plugged with cotton wool soaked in an antiseptic. If antiseptics are not available, a layer of quicklime may be put into the coffin and the corpse laid on it. Coffins should be closed immediately after putting in the body and should remain closed during transport and throughout the burial ceremony, which should be as short as local customs permit.

Mortuaries. The building should be fly-proof; floors, walls, and benches should be lined with materials that present even and smooth surfaces, which can be easily cleaned and disinfected; they should be kept scrupulously clean. Cholera victims should be placed in an isolated room, otherwise the precautions described in the preceding paragraph should be applied to all corpses, whether of cholera victims or not.

Washing facilities, with an abundant and safe supply of water, and ample drains for the rapid removal of waste water should be available in the room where bodies are prepared for burial. Equipment for the disinfection of the valuables of the victims and for the incineration of disposable materials should also be available within the premises. All objects in the hospital or house that have been soiled by or have been in contact with the patient's stools and vomitus during his illness should be treated in the same manner.

Ports of entry. To reduce the hazards of cholera importation from one country or region to another, strict control of sanitation facilities (water, sewage, wastes, food, and pests) must be exerted in places where travellers gather or are in transit, e.g., airports, ports, railway stations, bus terminals, and frontier control posts.

CONCLUSION

The measures described in this paper are of an emergency nature; their application will help in reducing the hazards to which countries threatened by cholera are exposed and in shortening the period during which transmission continues after an epidemic has begun. The effectiveness of these measures largely depends on the active collaboration of the people. A properly planned and organized campaign of public health education must be instituted from the very beginning to ensure the participation of everybody concerned.

It must be realized that improvisations are of very limited value; they should be considered only as palliatives to a situation whose correction demands long-term and costly solutions. Cholera, like other "filth" diseases, will not be wiped out without the construction and management of fully reliable sanitation works and services that guarantee the preservation of a healthful environment, and the improvement of the general living standards of the people.

List of Relevant WHO Publications and Documents

WHO Monograph Series[1]

No. 39: *Excreta Disposal for Rural Areas and Small Communities*, by E. G. Wagner & J. N. Lanoix (1958)
No. 42: *Water Supply for Rural Areas and Small Communities*, by E. G. Wagner & J. N. Lanoix (1959)
No. 48: *Milk Hygiene*, by various authors (1962)
No. 49: *Operation and Control of Water Treatment Processes*, by C. R. Cox (1964)

Other WHO Publications[1]

Guide to Hygiene and Sanitation in Aviation (1960)
Guide to Ship Sanitation, by Vincent B. Lamoureux (1967)
International Standards for Drinking Water (1963)

Documents[2]

WHO/CWS/RD/69.1: *The village tank as a source of drinking water*
WHO/CWS/RD/70.1: *Biological or slow sand filters*
BD/Cholera/70.3: *Cost-benefit and cost-effectiveness considerations in cholera control*, by K. K. Mathen
WHO/EH/70.1: *Cholera control through environmental sanitation*

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[1] See list of WHO sales agents on back cover.
[2] Available free of charge on request from: World Health Organization, 1211 Geneva 27, Switzerland.

CHAPTER 15

SURVEILLANCE AND CONTROL OF CHOLERA

KAREL RAŠKA[a]

Surveillance plays a very important role in the effective control of cholera at both national and international levels. It implies a continuous follow-up of the spread of cholera infection (in time and space) in individual countries and thoughout the world, and of all factors that might influence this spread. Systematic collection, analysis, and prompt dissemination of all epidemiological information are essential in order to facilitate epidemiological forecasts and the preparation of adequate control measures.

INTERNATIONAL SURVEILLANCE OF CHOLERA

The aim of the International Health Regulations (see Annex) is to ensure maximum security against the international spread of disease with minimum interference in world traffic. Recent experience has shown, however, that the existing Regulations do not constitute a sufficient deterrent to the introduction of cholera into a given country. It should be presumed that cholera can be introduced into any country in the world and efficient national epidemiological services will always be of primary importance in protecting the population against diseases so introduced. The quarantine barrier provided for in the previous International Sanitary Regulations represented a repressive approach, which has been only partially successful in the past. Very often countries took excessive measures which were an unnecessary hindrance to international commerce and travel.

It is expected that the global epidemiological surveillance programme will provide in the future a more technically oriented approach to the International Health Regulations.

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Close co-operation and exchange of epidemiological information between countries, especially between those that are infected and their non-infected neighbours, are of basic importance in the prevention of the international spread of cholera. Apart from the exchange of epidemiological information (particularly when cholera is spreading towards border areas), there are several other ways in which cholera-infected countries can help their neighbours.

It is evident that cholera spreads primarily through uncontrolled population movements by road and other routes of communication (coastal sea traffic), and much less through regular air or sea traffic. The migrations of nomads (the oldest type of international traffic) and other movements of population groups that occur for a variety of reasons (employment opportunities, pastures, religious pilgrimages) are also very important. The close co-operation of neighbouring countries and the co-ordination of health measures are therefore very desirable and in their mutual interest. Wars, fairs, festivals, and pilgrimages always greatly contribute to the spread of communicable diseases and especially of cholera.

Surveillance of cholera presents unusual difficulties because (*a*) areas of relatively poor hygiene, in which it tends to occur, are often lacking in adequate laboratory diagnostic facilities; (*b*) while the well-known acute disease is readily diagnosed on clinical grounds, the majority of infections are accompanied only by varying degrees of mild diarrhoea or are asymptomatic infections whose diagnosis is dependent upon laboratory findings; and (*c*) there is a disinclination to report cholera because of the now unjustified dread in which the disease is held.

WHO publishes in the Weekly Epidemiological Record the number of cases of cholera reported by Member States. The data do not reflect accurately the prevalence of the infection owing to variations in standards of reporting among different countries. Thus, some are based on the clinical diagnosis of hospitalized cases, others only on bacteriologically confirmed cases, and none of them include asymptomatic infections, not at least early in the invasion of an area by the disease. Consequently, even if it were possible to estimate the prevalence by using a correction factor based on the ratio of infected persons to acute cases, such data would not be comparable from one country to another. The data are also affected by the unfortunate tendency to conceal the presence of cholera, and the recent spread of the disease in the Middle East and Africa demonstrates the sequelae of such an attitude. The intentional concealment of the disease not only has long-term injurious consequences for the morale and quality of work of the epidemiological services, but also has a boomerang effect in loss of prestige and confidence among neighbouring countries. Nevertheless, unequivocal evidence of the

presence of cholera is invaluable for surveillance purposes, even though the true prevalence of the infection may not be known.

In the international field such an attitude complicates or even renders impossible the full implementation and results of international surveillance. It nullifies all efforts at international co-operation and the purpose of the International Health Regulations.

Over the years, WHO has developed a scheme of emergency aid in epidemics. Internationally recognized experts in the laboratory diagnosis, treatment, and epidemiology of cholera are ready, on request, to leave at very short notice (a few hours up to two days) for a country requiring assistance. They will provide any Member State with advice and, if necessary, material help such as vaccines, rehydration fluids, antibiotics, diagnostic sera, and other reference material.

In addition, WHO has organized periodical training courses for clinical and laboratory diagnosis, treatment, epidemiology, and prevention of cholera, and a WHO Reference Centre provides methodological advice and help. The recent history of cholera also gives many examples of the importance of such bilateral help to countries threatened by this disease.

In spite of the fact that cholera can be introduced into any country in the world, there are several examples of countries at risk that have successfully prevented the spread of cholera after its introduction by taking effective control measures (Japan, Hong Kong, and the USSR). It has been fully established that speed of action and the readiness of the health services to cope with the threat of a cholera outbreak (technically, by the availability of trained health personnel, and materially, by the provision of treatment and control facilities) are of the greatest importance in reducing losses, both in lives and economically.

NATIONAL SURVEILLANCE OF CHOLERA

Cholera thrives and spreads mainly in countries with low standards of sanitation and personal hygiene and poor basic health services. These countries are the receptive cholera areas, particularly if they border on cholera endemic foci or countries that are actually infected.

It is evident that travellers from cholera endemic countries may have a mild or inapparent cholera infection. So long as these persons do not have profuse diarrhoea and are living under good sanitary conditions, most instances are not recognized and the infection dies out. However, a different situation may arise if such persons stay in overcrowded households with insanitary living conditions. This explains why the index

case, or the actual moment of introduction of cholera, is often not recognized or reported for several days or weeks, particularly in countries with a relatively high incidence of diarrhoeal infections of varied etiology.

Surveillance also entails the optimum use of existing knowledge and facilities for the planning, implementation, and evaluation of control or preventive measures. It is essential, therefore, that sufficient trained personnel and stocks of material should be available to cope with the problem—e.g., to undertake the early detection and treatment of cholera cases and to interrupt the further spread of the infection.

It is extremely difficult to prevent the spread of cholera into a receptive country or to control the disease successfully and prevent it from gaining a foothold (i.e., becoming endemic) in a developing country. The well co-ordinated use of personnel and material resources, in numbers and quantities not normally available to the health services of most developing countries, is essential.

National anti-epidemic committee

A national anti-epidemic committee should be established under the chairmanship of the Minister of Health, and its members should include the Director of Health Services and an equivalent official responsible for medical supplies, as well as representatives of the Ministry of Defence, the Ministry of the Interior, and the Ministry of Finance. A clinician, a microbiologist, and an epidemiologist experienced in the field of cholera, together with a sanitary engineer and a health educator, should be invited to serve on the committee. Usually, the WHO country representative and a representative of the Red Cross or the Red Crescent are also invited to participate.

This committee can organize and co-ordinate the use of all available resources in a given task. In a large country or federation of states, subcommittees may be set up and normal channels of communication provided for co-ordination with the national committee. At the local level, village heads, community leaders, teachers, religious leaders, or other influential persons may be approached and asked for their cooperation in building a surveillance system.

Elements of surveillance activities

In a country at risk of the introduction of cholera, the following measures should be taken:

1. Consideration of the possible routes of introduction of infection into the country—by road, by sea, and by air. Health and customs personnel should receive detailed instructions for surveillance reporting, investigations, and control measures.

2. The surveillance and investigation of all suspected cases of severe diarrhoea with dehydration symptoms and deaths (with particular reference to children older than 4 years and adults) should be developed in all regions and districts. Efforts to isolate *V. cholerae* should be part of routine practice in laboratory investigations of all cases of diarrhoea.

Detailed instructions on cholera, its diagnosis, treatment, epidemiology, and control measures should be widely distributed to all medical personnel. Instructions in a simple form should also be sent to all paramedical staff, together with the necessary materials for collecting samples for laboratory investigation (transport media, etc.).

3. Training (or refresher) courses should be organized (if necessary, with bilateral or international assistance, e.g., from WHO) for clinicians, microbiologists, epidemiologists, and sanitary engineers.

4. Mobile teams (consisting of an epidemiologist, a clinician, an experienced nurse and, if necessary, a sanitary engineer or sanitarian) should be constituted and trained, both at central and at regional levels. Transport facilities (cars, boats, and helicopters) could be provided from the resources of the Ministry of Defence, the Ministry of the Interior, or the Security Services. The duties of these mobile teams are the immediate investigation of suspected cases or outbreaks, case finding, and emergency treatment and control measures.

They should therefore be equipped with apparatus for the collection of material for laboratory investigation, and with materials for the immediate application of treatment (rehydration fluids, antibiotics) and control measures (disinfection, vaccination).

5. A communication system should be established for the exchange of information between the periphery, the regions (regional mobile teams) and the centre. This system should operate on a 24-hour basis. The appropriate ministries should be asked to co-operate.

Any suspected case or outbreak should be immediately reported and investigated by the nearest (regional) mobile team and the results (negative, suspected, or positive) reported to the national committee. This is a most important part of surveillance activities and prompt and efficient action must be undertaken. Failure of the regional or central authority to take action when a suspected case of disease is reported inevitably demoralizes the whole surveillance system at the periphery. All persons arriving from infected areas without a valid vaccination certificate should be kept in isolation for five days.

6. All laboratories with facilities for bacteriological work should be able to isolate and identify *V. cholerae;* nutrient selective media, transport media, diagnostic sera, etc. should therefore be available. If possible, the best of these laboratories should be considered as the National Reference Laboratory which should provide all the other laboratories

with methodological guidance and reference material. Small clinical side-rooms in rural hospitals should be used only for the collection and despatch of material to the responsible laboratory.

7. Requirements with regard to the isolation and hospitalization of cases of cholera in different parts of the country, and the appropriate treatment facilities, should be considered and planned in advance.

Quite often it is difficult to utilize existing infectious disease departments or general hospitals, as these are already filled with patients having other serious illnesses. Therefore, the use of improvised hospitals (requisitioned buildings with satisfactory means of waste disposal), or emergency tent or hut hospitals (if climatic conditions permit), should be planned, with the help of the Ministry of Defence and other ministries. This also involves the need for special cholera beds, which are extremely important for a patient's care and rehydration (see Chapter 11).

8. An unexpected, large outbreak of cholera in a country could create serious difficulties with regard to the immediate provision of large amounts of rehydration fluids and antibiotics, which are vital for saving the lives of cholera patients. In countries where facilities exist for an extensive production of deionized or distilled pyrogen-free water and for sterilization (for example, in blood transfusion services), and that have competent staff to prepare rehydration fluids locally, it is more economical to import only the necessary chemicals, plastic bags, or glass bottles, tubing and needles. Rehydration fluids and antibiotics are needed in all countries, irrespective of whether they have cholera or not, so that there is no risk of losses in keeping adequate stocks of these materials. Therefore, one of the most important duties of the national committee is to arrange for these materials to be stocked in the central pharmacy. These supplies would be immediately available should an outbreak occur, and plans should be ready to increase the stock as required.

9. In view of the limited efficacy and duration of the protection provided by available cholera vaccines, priorities for their use should be established in the country at risk before an outbreak occurs, and the vaccination schedule agreed upon. A two-dose schedule is ordinarily used for individual immunization. However, one-dose schedules may be more practical for mass immunization. Immunization of the population should be carried out in accordance with the following priorities:

(*a*) *At national level*

(i) medical and paramedical personnel
(ii) customs personnel in ports and airports
(iii) public workers (postmen, public transport personnel, policemen, etc.)

(iv) population along mass travel routes (pilgrimages, etc.)
(v) all persons travelling abroad, as required by the International Health Regulations.

(*b*) *At local level*

(i) areas adjacent to countries already infected with cholera and coastal zones
(ii) important industrial enterprises
(iii) overcrowded camps and areas of large cities with poor sanitation.

If infection has occured, immunization of the exposed population should be carried out in a concentric pattern around the infected area.

10. An important part of cholera surveillance is to take adequate preventive measures with regard to all environmental factors that may facilitate the spread of cholera (co-operation with environmental health and food hygiene services). The following aspects deserve special attention:

(*a*) community water supplies
(*b*) production of ice and soft drinks (quality of water)
(*c*) sewage disposal, contamination of streams or sea-coast waters
(*d*) night-soil and its transport and disposal; communal latrines
(*e*) growing and fertilization of vegetables; source of water for "refreshing" them during their transportation and sale in the market
(*f*) production and distribution of different kinds of foods and fruits
(*g*) control of flies and preventing their access to human excreta.

Simple, cheap, but effective disinfectants (e.g., chlorinated lime, sodium hypochlorite in the form of laundry bleach) should be available in sufficient quantities for distribution at very low cost or free of charge for sanitation purposes. Simple instructions for routine disinfection and the principles of environmental and food hygiene should also be distributed to those concerned.

It should be stressed that water from uncontrolled sources should always be boiled, whether it is to be used for drinking purposes, for washing cooking and eating utensils, or for cleaning teeth.

11. Health education must be actively pursued. Even the best public health measures and sanitary facilities remain ineffective if they are not accepted by everybody or are not used properly and when they are not complemented by proper personal hygiene and practices.

The system of health education varies from country to country and is necessarily based on a thorough knowledge of the population and of their cultural background and habits. Health educators should avoid advocating any measures that cannot be carried out immediately, or

that may create panic and fear of the disease. Health education should be directed mainly to:

(i) obtaining the co-operation of the entire community through imparting an understanding of the measures necessary for preventing cholera and their follow-up;

(ii) improving the general standard of personal hygiene, particularly with regard to drinking and eating habits, and preventing faecal contamination of water sources and food.

A very important function of health education would be to enlist the aid of influential persons and muster material resources for surveillance activities and the immediate recognition and successful control of cholera in the case of its introduction into the country. These activities would, of course, be a serious challenge to the national committee.

12. Climatic or seasonal factors such as rains, monsoons, and drought may increase or diminish the incidence of the disease according to conditions in a given area. The role of these factors in a newly affected area should be carefully studied and the knowledge later applied in the planning of control programmes, as is done in endemic areas.

13. Provision should be made for the continuous exchange of epidemiological information and the co-ordination of preventive measures between neighbouring countries, whether affected by cholera or not.

National cholera control measures

Once cholera is introduced into a country a notification of every clinically diagnosed case of cholera should be telegraphed by the government within 24 hours to the World Health Organization. All clinical cases should be hospitalized; stool and, if possible, paired serum specimens should be sent to the microbiological laboratory; the patients should be given appropriate therapy.

All family or household contacts should be investigated, rectal swab or stool specimens taken for laboratory examination, and all contacts kept for 5 days under observation (quarantine). Prophylactic administration of antibiotics should be limited to family or household contacts for 2–3 days.

Careful attention should be given to the handling of dead bodies, which should be wrapped in blankets moistened with disinfectant (see Chapter 14).

Mobile teams should continue with systematic case finding, with the isolation and treatment of infected persons, and with the application of disinfection procedures and compulsory vaccination in and around the infected area.

Efforts should be made to trace the source and spread of the infection by examining rectal or faecal swabs from contacts, convalescents, or

mild diarrhoeal cases. During systematic epidemiological investigations all possible sources of infection should also be examined (drinking water, sewage, suspected vegetables or food).

The investigation of night-soil from households can be used in some areas for following up and tracing the circulation of *V. cholerae* in the population.

All isolated strains of *V. cholerae* should be identified and typed and representative strains sent to the WHO International Reference Centre for Vibrios. The collection and investigation of paired (acute and convalescent) sera from patients might be used for retrospective diagnosis.

The spread of cholera to new, virgin areas in Africa provides an excellent opportunity to investigate the value of serological surveys for agglutinating, vibriocidal, or other antibodies on population samples collected before and after the introduction of cholera. Such investigations should be made in co-operation with the WHO serum reference banks.

The epidemiological services should work in close co-operation with environmental health personnel (sanitary engineers) and health educators and should direct all their efforts towards localizing and interrupting the further spread of cholera in the country.

The surveillance of cholera is very difficult during the stage when the number of clinically manifest cases begins to decline. According to the International Health Regulations a cholera-infected area could be declared free of the disease when no case of cholera has appeared in the 10 days following the isolation of the last case. However, in reality surveillance in a given area should continue for several months, with a systematic search for even mild diarrhoeal cases and carriers, until the previously infected area or country can really be considered free of cholera.

In view of the ever-increasing extent and speed of international travel, and the practical impossibility of effective control of population movements between developing countries, the limitations of the International Health Regulations (see Annex) are obvious. It is clear that a great deal depends on the implementation of global surveillance activities and close co-operation between neighbouring countries.

A country can ask the World Health Organization for technical advice and material help at any time (emergency aid in epidemics). As always with the epidemiology and control of communicable diseases, help is easier and more effective the earlier it is requested. Requests for help should therefore be submitted as soon as possible after the recognition of the risk of introduction of cholera into the country.

Annex

SUMMARY OF THE INTERNATIONAL HEALTH REGULATIONS AS APPLIED TO CHOLERA

The World Health Organization is entrusted with the administration of the International Health Regulations[1] approved by the World Health Assembly. With regard to cholera, the Regulations contain several obligations for individual Member States. These are summarized below:

Each health administration shall notify the Organization by telegram or telex within 24 hours of its being informed that the first case of cholera, that is neither an imported nor a transferred case, has occurred in its territory and within the subsequent 24 hours shall notify the infected area. The existence of the disease, based on a reasonably certain clinical diagnosis, shall be confirmed as soon as possible by laboratory methods (Article 3).

This notification shall be promptly supplemented by adequate epidemiological information (source of infection, number of cases, number of deaths, and preventive measures taken) (Article 5). During an epidemic the notifications and epidemiological information shall be communicated to WHO at least once a week (Article 6).

An infected area may be considered free from infection when all measures of prophylaxis have been taken and maintained to prevent the recurrence of the disease or its spread to other areas, and when a period of time equal to at least twice the incubation period of cholera has elapsed since the last case identified has died, recovered or been isolated, and there is no evidence of spread of the disease to any contiguous area (Article 7).

The incubation period of cholera for the purposes of the International Health Regulations is considered to be five days (Article 62).

The possession of a valid certificate of vaccination (for a period of six months, beginning six days after one injection of the vaccine, or in the event of revaccination, within a period of six months from the date of that revaccination) should be accepted by all other countries. If a person possessing a valid certificate of vaccination against cholera has come from an infected area within the incubation period, he may be placed under surveillance for a period of not more than five days from the date of his departure from the infected area. If a person does not have a valid certificate of vaccination, he may be placed in isolation for the same period (Article 63).

A ship shall be regarded as infected if, on arrival, it has a case of cholera on board or if a case of cholera has occurred on board during a period of five days before arrival. A ship shall be regarded as suspected if a case of cholera has occurred on board during the voyage, but a fresh case has not occurred during a period of five days before arrival (Article 64).

An aircraft shall be considered as infected if, on arrival, it has a case of cholera on board. It shall be regarded as suspected if a case of cholera has occurred on board during the voyage but has previously been disembarked. However, a ship or aircraft coming from an infected area or having on board a person coming from an infected area shall be regarded as healthy if on medical examination the health authority is satisfied that no case of cholera has occurred on board during the voyage (Article 64).

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[1] The International Health Regulations were adopted by the Twenty-second World Health Assembly on 25 July 1969 and come into force as from 1 January 1971.

On the arrival of an infected ship or aircraft, all vaccinated passengers and crew members are put under surveillance upon disembarkation. Those not having valid vaccination certificates are isolated for five days. All belongings of sick persons and all contaminated articles are disinfected. Water carried on board and considered to be contaminated is removed and containers disinfected. Human excreta, vomitus and waste matter are disinfected before being unloaded or discharged from the ship or aircraft (Article 65).

Suspected ships or aircraft are subject to similar disinfection and all persons who disembark may be placed under surveillance for five days (Article 66).

After these measures have been taken the ship or aircraft is permitted to stay or to depart, i.e., is given free pratique (Article 67).

If a healthy ship or aircraft arrives from an infected area, those who disembark and who have valid vaccination certificates may be placed under surveillance for five days. All others may be isolated for the same period of time (Article 68). Those arriving by train or road are subjected to similar measures (Article 69).

The health authorities at the point of arrival may remove from an infected or suspected ship, aircraft, train, road vehicle or other means of transport coming from an infected area, samples of food (including fish, shellfish, fruit and vegetables) or beverages for culture examination, unless such food and beverages are in sealed packages and the health authority has no reason to believe that they are contaminated. They may prohibit the unloading of any of these articles found to be contaminated or may remove them (Article 70).

No person shall be required to submit to rectal swabbing. Only a person on an international voyage who has come from an infected area within the incubation period of cholera and who has symptoms indicative of cholera may be required to submit to stool examination (Article 71).

It is evident that the International Health Regulations concern the prevention of the international spread of cholera and other diseases covered by these Regulations. The World Health Organization as administrator of these Regulations has no jurisdiction over the internal affairs of Member States. The Organization does become involved when the provisions of these Regulations are not complied with.

Supplement

CHOLERA CONTROL

A Concise Review and Guide to Practical Measures

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This review has been compiled especially for those areas where cholera has been unknown for many decades and where the approach or the appearance of the disease would confront health authorities with unfamiliar problems.

EPIDEMIOLOGICAL FEATURES

A seventh pandemic of cholera appears to have developed since 1961, spreading from the South-West Pacific area into the Middle East in 1965 and 1966, and reaching North and West Africa and Eastern Europe in 1970. This pandemic is caused by the El Tor biotype of *Vibrio cholerae.*

Until about 1960, cholera was considered to occur only as an acute, often highly fatal infection, with purging diarrhoea. It is now clear that the infection is more often asymptomatic, or produces only a mild diarrhoeal disease closely resembling clinically that due to shigellosis, salmonellosis, *Escherichia coli* gastroenteritis, and similar infections. This is particularly true of the El Tor biotype, although it can also cause severe manifestations of cholera indistinguishable from those caused by classical *V. cholerae.* The El Tor biotype is slightly more resistant to various environmental factors and antibiotics, and survives longer in the environment, although the difference is not of epidemiological significance. It causes few secondary cases in families.

Cholera carriers may be incubatory, convalescent, contact, or, very rarely, chronic. Since the incubation period of cholera is short (1–5 days), incubatory carriage is of limited duration, but the convalescent carrier state may persist in a few persons for 2–3 weeks; the period of excretion of vibrios by the contact carrier is short, possibly 5 days to 2 weeks. Only a very few chronic carriers after clinical disease have been described. One such person, who has been found to harbour the vibrio in her gall bladder, has been excreting the organism intermittently for over 8 years.

Person-to-person transmission of the infection occurs, but by far the most important mode of dissemination is through the environment, especially water. In general, fomites appear to be of minor significance.

Seasonal incidence is characteristic within regions, but differs from one area to another, and epidemic cholera cannot be invariably associated with climatic factors in the general sense. There is no difference in incidence between the sexes or among age-groups, except that early in an epidemic in a newly invaded area there is a higher incidence

in adult males. This is taken to be due to the greater mobility of the male population and the resulting increased risk of exposure. In highly endemic areas, on the other hand, exposure early in life leads to the development of a continuously reinforced immunity, and the attack rate is highest in children. Although the incidence of cholera tends to be highest in the lower socio-economic groups, this appears to be attributable mainly to poor hygiene, and it has not been possible to associate nutritional deficiencies and similar factors with individual susceptibility to the disease.

CLASSIFICATION AND CHARACTERISTICS OF CHOLERA VIBRIOS

The cholera vibrio is a gram-negative, non-spore-forming, slightly curved rod which is actively motile by a single polar flagellum. It is not nutritionally fastidious, growing well in simple peptone water. It is aerobic, the optimum growth temperature is 37°C, and it is one of the most rapidly multiplying bacteria, outgrowing for example the coliform bacilli in the early hours of incubation. It is unusually tolerant of alkali, growing in media as alkaline as pH 9.2 ,a property sometimes utilized for purposes of primary isolation. It generally resembles the enteric bacilli in its tolerance of bile salts, etc.

Vibrios were separated by Heiberg into 6 fermentative types on the basis of the fermentation (producing acid only) of mannose, sucrose, and arabinose. The cholera vibrios usually, but not invariably, are Heiberg type I, fermenting mannose and sucrose, but not arabinose. Of other biochemical reactions, the formation of oxidase (oxidase test) and lysine decarboxylase, and the fermentation of mannitol (vibrio is positive in all three tests) are useful in the differentiation of *Vibrio* species, particularly NAG vibrios, from similar but unrelated species, such as *Enterobacteriaceae*, *Aeromonas*, and *Plesiomonas*.

The cholera vibrio is set apart serologically from other vibrios, in both its pathogenic forms, such as *V. parahaemolyticus*, and its non-pathogenic forms, by its specific O (heat-stable) antigen. It does, however, share heat-labile antigens with certain non-cholera vibrios; serological identification, therefore, depends upon the use of O-specific antisera. Three major O antigens can be differentiated within O-group I and have been designated A, B, and C. Of these, antigen A is considered to be the O-group I specific antigen. It occurs in combination with the other O antigens to give vibrio serotypes. The antigen combination AB is the Ogawa serotype, the combination AC the Inaba serotype, and the combination ABC the rare Hikojima serotype. Of these, the Ogawa and Inaba serotypes are of great practical importance. The relationship between these serotypes is still somewhat obscure, present evidence suggesting that the serotypes represent varying amounts of the antigens, as in the case of *Brucella*,

and that the Inaba serotype arises from the Ogawa serotype by antigen loss. There is some evidence also that spontaneous change in serotype occurs in nature and in experimental germ-free animals. Nevertheless, differentiation of the serotypes by the use of monospecific antisera, prepared by absorption with heterologous serotype antigen, is practical and has some epidemiological value.

The cholera vibrios, i.e., vibrios of O-group 1, are subdivided into biotypes on the basis of haemolytic properties, sensitivity to lysis by Mukerjee's group IV cholera phage at routine test dilution, sensitivity to polymyxin B, and agglutination of chicken or sheep erythrocytes. Of these differentiable characteristics. haemolytic activity appears to be relatively unstable.

Haemolytic vibrios of O-group 1 (vibrios of other O-groups may be haemolytic also) were isolated at the El Tor quarantine station in Egypt in 1905 and were designated as El Tor vibrios. Prior to 1938 they were considered to be non-pathogenic with respect to cholera in man. Pathogenic, cholerigenic El Tor vibrios found in that year in Sulawesi have subsequently spread widely as the etiological agent of the present pandemic of cholera.

While the El Tor vibrios of O-group 1 were originally defined on the basis of haemolytic activity, they now seem to be losing this property, as for example in the Philippines, although the vibrios continue to be called El Tor. Haemolytic activity can, however, still be detected in most strains by using sensitive techniques.

CHOLERA PHAGES

Cholera phage was first described in 1918 by d'Herelle, and since 1920 it has been known that it often coexists with susceptible cholera vibrios in the patient. This results in the appearance of "nibbled" or "moth-eaten" colonies on primary isolation plates, provided that the culture medium does not contain a phage-inhibitory agent such as bile salt. Since the discovery of the temperate phages it has been known too that almost all strains of the classical biotype of *V. cholerae* and many of the El Tor biotype isolates are lysogenic. Phage-typing of the cholera vibrios initially studied by Asheshov has not proved as useful as phage-typing of some other species of bacteria, but Mukerjee has developed 4 groups of cholera phages which permit the classical biotype to be divided into 5 types, and 5 groups of El Tor phages by means of which the El Tor biotype can be subdivided into 6 types.

Mukerjee has also described group IV cholera phage as being a means of differentiating the classical vibrio biotype from the El Tor strains, even though the latter may deviate from the typical pattern in some characteristics, especially haemolytic activity. The classical

biotype is sensitive to, or lysed by, phage IV at routine test dilution, while the El Tor biotype is not. Phage-sensitivity with respect to phage IV is thus one of the most valuable, and possibly one of the most stable, of the characteristics used in the differentiation of the cholera vibrio biotypes.

The earliest studies on cholera phage were motivated largely by its possible therapeutic value. The results of such studies were invariably disappointing. Interest in the possible value of the phage therapy of cholera was therefore quiescent until recently (1963), when it was revived by Russian workers. Encouraging results were reported for both phage prophylaxis and therapy. However, recent studies in East Pakistan by Soviet investigators in collaboration with American and Pakistani scientists have failed to confirm these observations, and it appears that phage therapy of cholera is of almost no value.

LABORATORY DIAGNOSIS

1. Collect specimen by means of a rubber catheter, glass rod, blunt glass tube with an eye, rectal swab, or faecal swab from the faeces passed in any container.

2. Transport specimen in Venkatraman-Ramakrishnan fluid, Cary-Blair medium, alkaline peptone water, or taurocholate tellurite broth, or on blotting paper strips.

3. Enrich in alkaline peptone water. Incubate at 37°C for 6–8 hours.

4. Plate by streaking the specimen after enrichment directly on TCBS agar and nutrient agar, or TCBS agar and bile salt agar, or TCBS agar and gelatin agar. Incubate overnight.

5. Perform primary slide agglutination with polyvalent (group O) serum.

6. Isolate on Kligler iron agar or agar slant. Incubate overnight.

7. Confirm slide agglutination with polyvalent and typing sera:

A. If positive, the following tests should be done to determine the Heiberg group and the biotype of the vibrio:

(*a*) Fermentation of mannose, sucrose and arabinose

(*b*) Haemagglutination with chicken or sheep red cells

(*c*) Tests for resistance to polymyxin B using a 50 μg disc or 15 μg/ml in the medium

(*d*) Test for resistance to cholera phage group IV at routine test dilution

(e) Test for haemolysis by growing the organism in nutrient broth and heart infusion broth.

Cholera vibrios of both biotypes usually belong to Heiberg group I, being mannose and sucrose positive and arabinose negative. Biotypes are characterized by:

	Classical	*El Tor*
Direct haemagglutination:	negative	positive
Resistance to polymyxin B:	sensitive	resistant
Resistance to cholera phage group IV:	sensitive	resistant

B. If negative, the organism may be a non-agglutinable or a non-cholera vibrio but should be differentiated from the closely allied species by doing the tests for oxidase, lysine and ornithine decarboxylase, arginine diydrolase, fermentation of mannitol, and the oxidation-fermentation test (see Tables 1 and 2, Chapter 5).

ISOLATION OF CHOLERA VIBRIOS

1. *From water*

Samples should be collected in a sterile 1-litre bottle. The bottle should contain 2 teaspoonfuls of common salt before being sterilized in an autoclave. After the water sample has been collected, the pH may be adjusted to 9.2 with N/1 NaOH before transport to the laboratory, if the laboratory examination is likely to be delayed.

(*a*) *Simple filtration technique.* 300–1000 ml of the water sample are passed through a Seitz filter (6-cm disc) under slow suction. The disc is then folded and placed in 50 ml of alkaline peptone water in a wide-mouthed vial or flask, the pH is adjusted to 9.2 and the culture incubated at 37°C for 6 hours before being plated on suitable media.

(*b*) *Millipore filter method.* A millipore filter disc fitted to a millipore filter holder is used. After filtering about 1 litre of the water sample, the filter paper is treated as described in (*a*) or the disc is put on the surface of a suitable selective solid medium and the colonies are counted under the dissecting microscope after incubation for a minimum period of 6 hours.

(*c*) *By double enrichment in peptone water.* 280-ml bottles containing 20 ml of 10% peptone water with 5% of NaCl are sterilized and sent to the field for collection of water. 200 ml of water are collected in each bottle and despatched to the laboratory. The pH is adjusted to 9.2 and the contents of the bottle incubated overnight; 1–2 ml are then transferred to 10 ml of alkaline peptone water to be incubated for 6 hours before being plated out.

The peptone water enrichment method appears to be quite suitable and effective for use in ordinary laboratories. In experienced hands, quantitative results may be obtained by the millipore filter technique.

2. *From sewage*

The sewage sample is diluted with sterile saline so that it can be filtered through sterile gauze. The filtrate is treated in the same manner as a water sample.

3. *From foodstuffs*

Random samples are collected from the exposed parts. These are triturated in alkaline peptone water and then treated in the same way as water samples. Liquid foods are filtered and treated as described for water.

4. *From fomites*

Washings from the exposed surfaces are filtered and treated as described for water.

5. *From flies*

(*a*) *From external surfaces.* A pooled collection of flies from a given locality is washed with alkaline peptone water, which is then incubated for 6 hours and plated out.

(*b*) *From interior of the gut.* A pooled collection of flies is rinsed with 70% alcohol, washed thrice with sterile saline, crushed in alkaline peptone water and treated as above.

PATHOGENESIS

Infection of man with the cholera vibrio occurs by the oral route through the ingestion of water or food contaminated with *V. cholerae.* Since the vibrios are acid-sensitive, it seems unlikely that they can reach the small bowel in sufficient numbers to establish a focus of infection unless the barrier of gastric acidity is temporarily lowered. As the vibrios multiply, they elaborate and release an exotoxin. The manner in which this toxin affects the secretory mechanisms of the bowel tissues is not fully understood, but it is known to induce or accelerate the movement of water and ions from the tissues into the lumen of the gut to give a net secretion of isotonic fluid manifested as a watery diarrhoea. There is no histological evidence of any damage to the capillaries or any other tissues in the gut. Recent studies have also indicated that reabsorption of sodium and water is not impaired in cholera and that there is no inhibition of the sodium pump as was thought previously.

Massive gastrointestinal loss of isotonic fluid of very low protein but high bicarbonate and potassium content leads to dehydration with haemo-concentration, hypovolaemic shock, and metabolic acidosis because of the drain of bicarbonate. These events, if they proceed long enough, also lead to impairment of renal function, resulting in increasingly severe acidosis. The loss of potassium is less serious in the adult and the impairment of renal function, in fact, tends to conserve potassium. Hypokalaemia is more serious in paediatric cholera, and may become sufficiently severe to cause paralytic ileus and cardiac arrhythmia; replacement of potassium by its inclusion in the oral or rehydration fluid is therefore required.

MANAGEMENT OF CHOLERA PATIENTS

Cholera cases should be isolated and treated in a separate ward of an infectious diseases hospital. Arrangements should be made beforehand where there is a risk of an epidemic. In a rural area, a school building or tents may be suitably converted into a hospital.

Essential requirements

1. Rehydrating fluid with disposable administration set
2. Weighing machine
3. Cholera cot with graduated bucket
4. Antibiotic
5. Intake-output chart

Precautions to be taken by hospital staff

1. Although laboratory or hospital infection is extremely rare, the staff must observe the basic principles of personal hygiene.
2. The staff should be vaccinated against cholera; they need not take antibiotics.
3. Doctors and nurses should wear white coats in the cholera ward, and discard them before leaving the ward.
4. Eating and drinking by the staff in the ward must be avoided.
5. Hands should be washed carefully with soap and disinfectants after touching patients and their clothes.

Treatment of cholera in adults and children weighing more than 20 kg

1. A brief clinical examination of the patient should be made to avoid misdiagnosis.
2. Body weight should be taken, if possible, as the quantity of fluid required for initial rehydration of patients with severe diarrhoea and collapse may be as much as 10% of their body weight.
3. Place the patient on a cholera cot with a bucket underneath. If the patient has to be treated on the floor, an earthen container may be placed in a hole under his buttocks.
4. Take a rectal swab or stool specimen for laboratory examination.
5. Commercially available lactated Ringer's solution is considered to be the most convenient fluid for the treatment of cholera for both initial rehydration and maintenance.
6. Initial rehydration. Intravenous rehydration should be started as soon as possible with an 18-gauge needle. About 1 litre of the fluid should be administered in the first 15 minutes, and then 1 litre every 30–45 minutes. 2–5 litres of fluid may be required for the initial rehydration.
7. Antibiotic therapy with tetracycline (500 mg every 6 hours for 2–3 days) should be started as soon as vomiting ceases (usually after 3 hours).

8. Maintenance of rehydration. The stool output should be measured every 6–8 hours and intravenous fluid given to match the volume lost and so maintain the water-electrolyte balance. The same result can be attained by giving an oral maintenance fluid (glucose 21.6 g, NaCl 3.50 g and sodium bicarbonate 2.52 g per litre of drinking water). 1.5 volumes of this fluid should be given by mouth for each one volume of stool lost during the preceding 6 hours.

9. An accurate fluid intake and stool output chart must be maintained.

10. If the fluid replacement is adequate, blood pressure, pulse and skin elasticity return to normal, the patient feels comfortable, and urine output is re-established. Determination of plasma specific gravity is helpful but not essential.

11. Patients receiving intravenous maintenance therapy should be encouraged to drink moderate amounts of water, tea, or green coconut water, the latter being a good source of potassium.

12. Normal diet may be allowed as soon as the patient desires.

13. Fluid replacement should continue until diarrhoea ceases. When the patient is treated with antibiotics, hospitalization may not be necessary for more than 3 days.

14. Replacement of potassium is not essential in adults. If necessary, 10 ml of a solution containing 100 g each of potassium acetate, potassium citrate, and potassium bicarbonate may be given 3 times a day. Alternatively, for each litre of stool lost 170 ml of green coconut water may be given, when available.

15. Complications such as pyrogenic reaction, persistance of acidosis and dehydration, renal failure, acute pulmonary oedema, hypokalaemia, and over-hydration should not occur in a properly treated, uncomplicated case of cholera, and case fatality should be about 1% or less.

Treatment of cholera in young children

1. Children below 5 years of age (less than 20 kg) need greater attention as they do not stand dehydration well and they are prone to develop complications such as hypoglycaemia, convulsions, cerebral oedema, and paralytic ileus. The sodium content of the stool is lower in children with cholera than in adults. At the same time, rehydration is technically more difficult.

2. Careful recording of body weight, stool output and fluid intake is essential. During treatment, a four-hourly watch must be kept on the pulse rate, pulse volume, blood pressure, skin elasticity, level of consciousness, facial oedema, lung fields, and urine output in order to evaluate the adequacy of fluid replacement.

3. Fluid may be administered through the scalp veins using special paediatric scalp vein sets, or through the jugular or femoral vein in very severely ill children. Short, small-gauge needles (No. 22 or 24) are helpful.

4. Ringer's lactate has been found to be the most convenient and satisfactory fluid.

5. Initial rehydration. A severely ill child in shock usually has a fluid deficit of 10%. A hypotensive child with normal sensorium has a fluid deficit of about 8%, and a case of mild diarrhoea with diminished skin elasticity and tachycardia but normal sensorium has a fluid deficit of about 5%. The volume of fluid required in millilitres may be roughly estimated by multiplying the body weight in kg by 10 times the fluid deficit in per cent.

6. Antibiotic therapy with tetracycline (50 mg/kg/day) should be started as soon as vomiting ceases. The antibiotic should be given in 4 divided doses for 2–3 days in the form of a liquid suspension.

7. Maintenance of rehydration is ensured by intravenous administration of Ringer's lactate, but the volume should be approximately 25% less than the stool output. Oral fluid has been found by some clinicians to be less satisfactory than in adults.

8. A liberal amount of 5% glucose in water should be given by mouth as soon as vomiting ceases.

9. Potassium replacement is more important for children. It may be carried out in the same way as in adults.

10. Too rapid fluid administration should be avoided as it may cause convulsions. Prolonged stupor may be a sign of hypoglycaemia and can be prevented by adding glucose to the oral or intravenous fluid. Over-hydration should be avoided by judicious use of the fluid.

IMMUNE RESPONSES IN CHOLERA

Recovery from cholera is associated with a serum antibody response manifested as agglutinin and vibriocidal antibody. It has been reported also that convalescent sera contain antibody to the vascular permeability factor (PF) demonstrable by neutralization of the intradermal reaction to cell-free preparations. In addition, these sera contain antibody that neutralizes the enterotoxin associated with the movement of water and ions from the tissues into the lumen of the bowel. The role of PF in the pathogenesis of the disease and its identity or non-identity with the enterotoxin are at present controversial.

Serum antibody titres decline relatively rapidly on recovery, making retrospective serodiagnosis of questionable value after some weeks. In areas where brucellosis is present, the occurrence of common antigenic factors between the cholera vibrio and *Brucella* complicates the interpretation of serum agglutinin and vibriocidal antibody titres in other than paired sera. Since anti-PF antibody appears to be formed in response to infection, but not to immunization with present vaccines, it is considered by some to be of value in differentiating the immune response to vaccine from that to infection.

A variety of toxic substances are formed by the cholera vibrio; these have been classified into three groups on the basis of heat stability and dialysability, and designated types 1, 2 and 3. Type 1 toxin(s) is heat-stable (to 100°C) and non-dialysable. It has a high carbohydrate content and is considered to represent a Boivin-type endotoxin which is lethal to mice and cytotoxic in chick embryo and tissue culture. Type 2 toxin(s) is heat-labile (56°C for 10 minutes) and, in the crude state, does not pass through cellophane dialysis membranes or membrane filters having a molecular weight exclusion of 10 000. It is an exotoxin which diffuses freely from the intact bacterial cell in contrast to type 1 toxin(s) whose liberation in soluble form results from dissolution of the bacterial cell. Type 2 toxin includes the diarrhoea-producing toxin, or enterotoxin, and PF activity. Type 3 toxin(s) is heat-stable (100°C), diffuses freely from the bacterial cell, and is readily dialysable. The inhibition of sodium transport in frog and toad epithelium by type 3 toxin(s) is now known to be an artifact attributable to the presence of the ammonium ion.

The occurrence of asymptomatic infections suggests the relative importance of antitoxic immunity to the diarrhoea-producing toxin in cholera, i.e., the presence of an antitoxic immunity at a sufficiently high level to prevent diarrhoeal manifestations of the infection. It is therefore believed that an effective immunity to cholera is dual in nature, and includes both antibacterial and antitoxic components, the former presumably serving to inhibit or prevent colonization of the small bowel, and the latter neutralizing the activity of the diarrhoea-inducing toxin.

CHOLERA VACCINES

Cholera vaccine consists of a saline suspension of killed cholera vibrios containing about 8000 million bacteria per ml. The killing and preservative agent is commonly phenol, but formaldehyde and organic mercurials, such as thiomersal, may be used, or the vibrios may be killed by mild heat (56°C for 30 minutes). The vaccine is bivalent, containing equal numbers of the Ogawa and Inaba serotypes. The vibrio is usually of the classical type, but the El Tor biotype has also been used. Classical vibrio vaccine appears to protect equally well against both types of infection. Vaccines are standardized by the mouse-protection test, but when this is impractical the antibody (agglutinin, vibriocidal antibody) response of rabbits is acceptable. Immunization is preferably carried out by subcutaneous or intramuscular inoculation of two doses, 0.5 and 1.0 ml, 7–28 days apart, and reduced doses, 0.1–0.3 ml, for children under 10 years of age. Single doses of 1.0 ml may be used for mass immunization under epidemic conditions, and may be nearly as effective in an endemic area as the two-dose regime.

Present cholera vaccines stimulate an antibacterial but little or no antitoxic immune response. Such vaccines have been shown in field trials to produce an appreciable, though variable, degree of protection against naturally-occurring infection, indicating that antibacterial immunity plays a significant part in effective immunity to the disease. Corresponding evidence concerning antitoxic immunity in man is not yet available, but such immunity has been shown to be associated with protection against challenge infection in the canine model of experimental cholera.

Vaccine-induced immunity is of limited duration—of the order of a few months—and is considered not to persist longer than 6 months at the most. The efficacy and duration of the immunity presumably associated with recovery from the naturally-acquired disease is less clear in view of the low attack rate and consequent small probability of a second attack. Although cholera vaccine confers only partial and temporary protection, its use is justified in epidemic situations, in conjunction with other public health measures, to reduce the number of cases.

ENVIRONMENTAL HEALTH MEASURES

The most important single vehicle for the spread of cholera is water. Water supplies for urban centres require inspection of catchment areas, water courses, wells, springs, and reservoirs to prevent initial contamination from sewer and septic tank discharges and defective latrines, and from indiscriminate defecation and insanitary disposal of excreta. It is best to remove all dwellings from the area and, in the case of streams, to direct the discharge of pollution downstream. Streams require strict surveillance for at least 500 m upstream and 50 m downstream from the point at which water is taken. The distribution system must be subject to continuous inspection to avoid contamination through leaks, and pressure must be maintained to prevent backflow, particularly at the periphery of the system. Sampling for chlorine, to ensure adequate concentrations, should also be done at outlying points, and during an emergency the chlorine dosage should be increased from ample stocks built up in advance. The usual free chlorine concentration of 0.2–0.4 mg/litre is not sufficient in emergencies, and an increase in dosage is strongly urged, in spite of possible objections from the public. Emergency chlorination can be applied at booster pumping stations or other convenient points. When sufficient chlorine gas is not available, solution-feed chlorinators may be used. Water supplies in slum areas are commonly deficient; such supplies and water supplied to public places must be subjected to rigorous control, including chlorine analysis and bacteriological

examination, since such areas can be particularly dangerous for the spread of infection. Ice and bottled waters require the same precautions. Bacteriological control is essential.

Water supplies in rural areas are more difficult to control. Dug wells may be disinfected with chlorine using the pot method by which a double cylindrical pot containing a mixture of sand and bleaching powder is lowered into the well to a point 1 m or more below the surface of the water. Such a device is satisfactory for 2–3 weeks in small household wells, but will need to be renewed if the emergency continues beyond that time. Drilled wells and springs are subjected to strict inspection, with particular reference to cracks and other leaks into which contaminated surface waters may penetrate. Surface waters present particular difficulties owing to the facility with which they may become contaminated, and they are the least desirable sources of potable water. An effort should be made at least to treat that portion of the water used for drinking and cooking, either by chlorination if bleach is available, or by boiling prior to use. Particular attention must be paid also to the conditions under which water for such purposes is stored. Portable water treatment plants can be used in emergencies, but are only temporary substitutes for hygienic measures. Such plants should in any case be maintained in readiness, and it is wise to provide them for emergency use in areas repeatedly exposed to epidemics. Truck or trailer tanks equipped with pumps are also valuable in emergencies, but clearly can supply clean water on only a relatively small scale and for a short time.

The provision and maintenance of a properly and efficiently functioning sewerage system is second only to water supply control in the prevention of cholera. Human excreta are the only source of contamination of water supplies by cholera vibrios and the disposal of sewage in such a manner as to avoid mass contamination of otherwise potable waters is a matter of primary importance in urban centres where the quantity of excreta to be disposed of necessitates a sewerage system and appropriate treatment of the sewage. The absence of any treatment whatsoever and the consequent dumping of large amounts of raw sewage into, for example, a stream that serves as a water supply to persons living downstream can markedly facilitate the widespread dissemination of cholera infection. Any treatment of sewage is therefore preferable to none.

While it is true that the cholera vibrio cannot compete successfully with saprophytic micro-organisms, it is well established that even completely treated sewage may require chlorination in order to ensure freedom from vibrios. Sewage used for irrigation purposes may lead to the contamination of otherwise potable waters as well as of vegetables and other foods that are often consumed in the uncooked state. Similarly, the use of night soil as a fertilizer constitutes an even more serious source of cholera infection, since it is not diluted and in addition may contaminate the environment from the spilling of buckets and

tanks used for collection. The collection and use of night soil for this purpose should be avoided in cholera-infected areas.

In the absence of sewerage systems, as in rural areas, excreta should be disposed of in suitably located latrines. These must not only be sited downhill of any water supplies, such as wells, but must also be sited with due consideration for the nature of the soil. Thus, in stony areas cracks and fissures, particularly in limestone, can result in contamination, not only of dug wells but also of drilled wells. Latrines should also be periodically disinfected with chlorinated lime or similar chlorine-liberating substances.

Special attention must be directed to the disposal of excreta in places such as hospitals, markets and restaurants, for the dissemination of infection in these places can result in widespread disease. It is desirable also that foods and places in which foods are sold be subjected to strict supervision.

SURVEILLANCE AND CONTROL

Surveillance of cholera is difficult because most countries affected by this disease do not have adequate basic health and laboratory services. Moreover, infection with *V. cholerae* causes a very high proportion of asymptomatic infections and cases of very mild diarrhoea. In spite of this, all cholera-affected countries do carry out some kind of surveillance. Even though basic information on the prevalence of cholera tends to be fragmentary, it has now been established that during the period 1961-1970 the present pandemic has developed through spread of the infection from the South-West Pacific and South-East Asia, through Afghanistan and the Middle East to southern USSR, Africa and Europe (see Chapter 2). Only continued surveillance will allow the detection of its further spread and the intelligent application of preventive and control measures. It has also been demonstrated that the introduction of cholera into a country cannot be prevented, but its spread within a country can be limited by improving sanitation and by undertaking intensive surveillance. The enforcement of strict surveillance measures is particularly important for developing countries threatened by cholera and will help them to detect the infection before it becomes widespread and out of control. Prompt notification of outbreaks will permit the World Health Organization to serve a very useful purpose by informing Member States of the true epidemiological situation in the cholera-affected countries.

Preparatory or alert phase

Countries in which cholera is endemic or that are threatened by cholera should establish national and provincial cholera surveillance and control committees, whose members should include executive officials and technical experts from appropriate ministries and represen-

tatives of the Red Cross (or Red Crescent) and other voluntary agencies. The tasks of these committees should be to make policies and direct various activities within the country and to co-ordinate international and bilateral assistance.

The committees of a country threatened by an approaching outbreak should consider the possible routes of introduction of infection and should ensure that in vulnerable areas laboratories are supplied with necessary equipment, chemicals, diagnostic media and sera, and that treatment facilities, with cholera cots, rehydration fluids and antibiotics, are made ready. They should also arrange for teams of bacteriologists, epidemiologists, clinicians and sanitary engineers or sanitatarians to be trained in anti-cholera measures and should set up peripheral or field posts or mobile teams equipped with transport media, etc. for the collection of information and of samples.

To derive maximum benefit from cholera vaccines, it is essential they be used with prudence (see Chapter 13). Vaccination should not be used as a political weapon, but only to reduce the number of acute cases in the face of an epidemic. When there is a cholera threat, it is advisable to vaccinate the population bordering the infected territories and in coastal zones, overcrowded camps, and areas with poor sanitation. Medical and paramedical personnel likely to be exposed, travellers, customs officials, etc. should also be vaccinated. In endemic countries, previous experience should indicate which sections of the population and which areas are most vulnerable and should be given priority. The preparatory stage is the best period for arranging for safe water supply and proper disposal of night soil, and for disseminating information to the public on the methods of spread of cholera and means of prevention. Emphasis should be on improving personal hygiene and sanitation and not on vaccination, which is of limited value. Attention should also be paid to insanitary practices peculiar to certain localities, such as the use of night soil as a fertilizer.

Private practioners and public health officials should be alerted to the situation and given technical information. Arrangements for the transportation of specimens and of teams and patients should be made, and a system of communication should be established for co-ordinating all the activities, e.g., among the field epidemiologists, laboratories, hospitals, and the central co-ordinating committee. Supplies of vaccines, rehydrating fluid, giving sets, disposable syringes, needles, antibiotics, laboratory chemicals and reagents, and disinfectants should be ensured. Pedojets with special nozzles for intramuscular injections are useful if experienced personnel are available to use them.

The national committee may arrange for assistance from the World Health Organization for procuring essential supplies of equipment, chemicals, vaccines, rehydrating fluid, etc., either on a reimburseable basis or as a donation. Requests may also be made for assistance in training national medical and paramedical personnel; the granting

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of such requests will be facilitated if WHO is kept informed of the true epidemiological situation regarding cholera within the country.

Countries with facilities for making rehydrating fluid should increase their production. Technical assistance and supplies for starting production of rehydrating fluid can be requested through WHO.

Invasion period

The positive finding of a case of cholera should lead to immediate notification, hospitalization of the affected person, bacteriological examination of the family and household contacts, prophylactic administration of antibiotics to close contacts (who should be isolated in their homes if possible), vaccination of the population of the infected area following a concentric pattern, and the detection and isolation of new cases and their contacts.

After discovery of the first case, efforts should be made to trace the source of infection by proper epidemiological investigation and, if possible, the source should be removed. It is of the greatest importance to ensure a safe and sufficient water supply and proper excreta disposal, and proper attention should be paid to health education of the public to improve personal and food hygiene. Disinfectants should be made freely available for disinfection of latrines, drains, and infected houses and their surroundings. All public gatherings and feasts should be prohibited; where this is difficult out of respect for local social customs and rituals, assistance should be provided to prevent the spread of infection. Dead bodies should be handled with special care, and the clothing and utensils of the sick should be disinfected or destroyed.

For several months after the epidemic is over, all cases of diarrhoea should be investigated bacteriologically for vibrios, in order to detect any new cases and contacts, who should be isolated and treated.

WHO has developed a scheme of emergency aid in epidemics. Internationally recognized experts in the diagnosis, treatment and control of cholera are ready, on request, to leave at very short notice for a country requiring assistance.

Long-term plans

It is imperative for all the countries affected or threatened by cholera to undertake long-term plans to improve the sanitation and water supply in order to dispel the threat of cholera, if necessary with bilateral and multilateral assistance. Experience has shown that slight improvements in sanitation and water supply markedly reduce the transmission of *V. cholerae*, which is a rather delicate organism.

WORLD HEALTH ORGANIZATION
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PRINCIPLES AND PRACTICE OF CHOLERA CONTROL

CORRIGENDA *

Page 7, lines 4 and 5
delete: existing International Sanitary Regulations, as at present applied, have proved
insert: International Sanitary Regulations that were in force until 31 December 1970 were

Page 10, line 27
delete: Sanitary Regulations [1] currently in force
insert: Health Regulations (1969) [1]

Page 10, footnote
delete: Revised Regulations, to be known as *International Health Regulations*, will come
insert: These Regulations came

Page 17
replace figure by new Fig. 1 attached

Page 18, line 1
delete: at least
insert: more than

Page 18, line 6
delete: Leona
insert: Leone

Page 18, line 7
delete: Oman
insert: Sheikhdoms

Page 18, list of territories
add: Dahomey, Ethiopia, France, Gaza Strip, Ghana, Mali, Nigeria, Somalia, Togo

Page 18, Fig. 2
replace figure by new Fig. 2 attached

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* Some of these corrections have been necessitated by the coming into force of the new *International Health Regulations* on 1 January 1971. Furthermore, the rapid extension of the cholera pandemic during the last months of 1970 has rendered some of the epidemiological information given in Chapter 2 out of date; the situation as known to WHO at the end of January 1971 is reflected in the two new maps attached and in the corrections to page 18. A number of other amendments that have been suggested since publication of the book are also listed.

Page 19, line 3 of title
delete: 1965-1970
insert: 1965 to October 1970

Page 31, line 24
delete: observations
insert: experiments and observations

Page 65, line 11
delete: et al.,
insert: & Phillips,

Page 71, line 12
delete: (in press)
insert: **45**, 374

Page 71, line 19
delete: et al.
insert: & Phillips, R. A.
delete: 199
insert: 999

Page 107, line 27 (heading)
delete: SPECIAL
insert: OTHER

Page 107, lines 28-31
delete

Page 107, line 33
delete: of particular importance
insert: important

Page 107, lines 34 and 35
delete: considered as foci of infection and treated accordingly ; they should be

Page 107, lines 35 and 36
delete: and protected

Page 108, lines 3 and 4
delete: and wrapped in shrouds that have been soaked in an antiseptic preparation

Page 108, lines 5 and 6
delete: All the body orifices should be plugged with cotton wool soaked in an antiseptic.

Page 109, line 8 (heading)
delete: and Documents

Page 109, lines 20-25 and footnote 2
delete

Page 111, lines 13 and 14
delete: the existing Regulations do not constitute a sufficient deterrent to
insert: such Regulations do not offer complete protection against

Page 111, lines 18 and 19
delete: previous International Sanitary Regulations
insert: International Sanitary Regulations previously in force

Page 115, lines 37 and 38
delete: without a valid vaccination certificate should be kept in isolation
insert: should be kept under surveillance

Page 116, line 32
delete: priorities
insert: the necessity, if any,

Page 116, line 33
delete: the
insert: a possible

Page 118, line 31
delete: (quarantine)

Page 118, line 33
delete: 2-3
insert: 3-5

Page 118, lines 34-36
delete entire sentence
insert: Due care should be taken in the handling of dead bodies.

Page 119, line 24
delete: Regulations
insert: Regulations (1969)

Page 120, footnote, line 2
delete: come into force as from
insert: came into force on

Page 132, line 22
delete: 10 ml

Page 132, line 23
delete: may be given 3 times a day.
insert: per litre may be given 3 times a day in 10-ml doses suitably diluted

Page 138, line 19
delete: the population
insert: selected populations

FIG. 1. EXTENSION OF CHOLERA 1961-1970

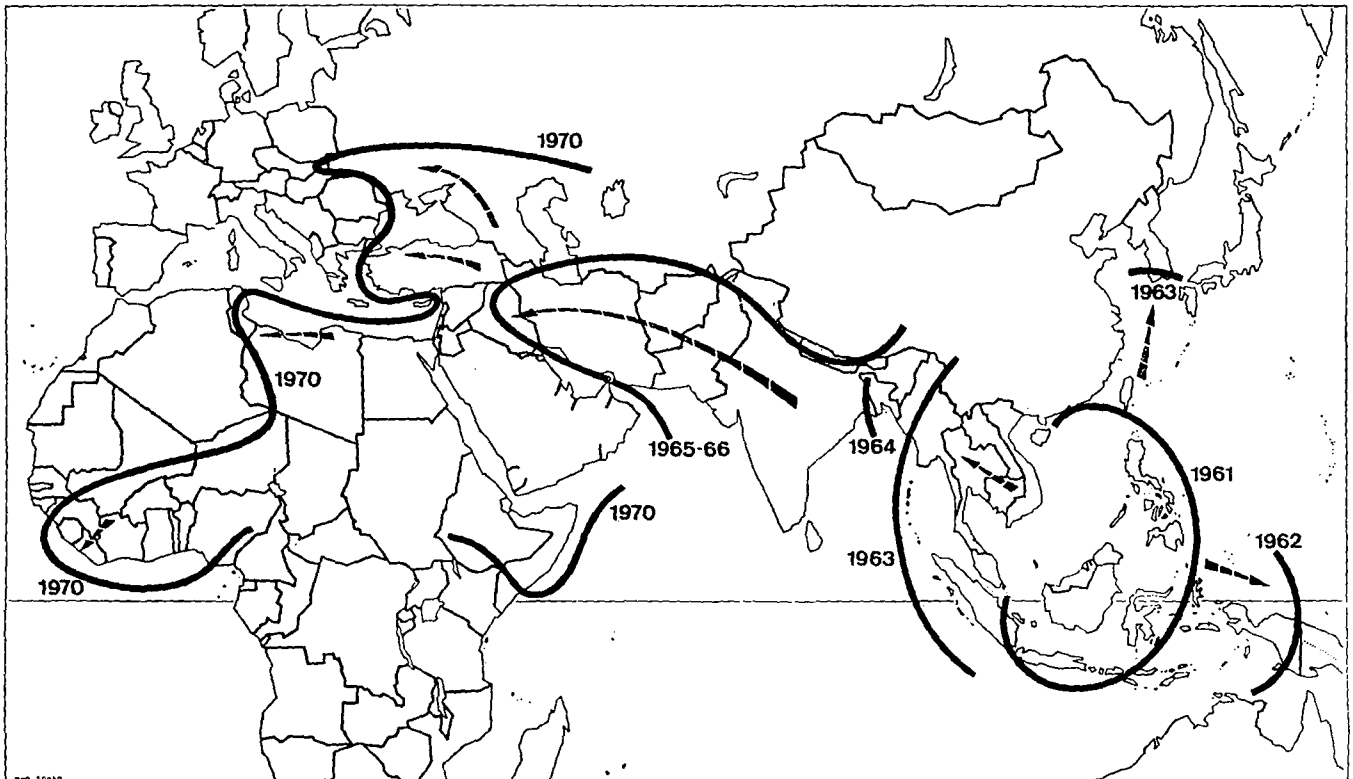
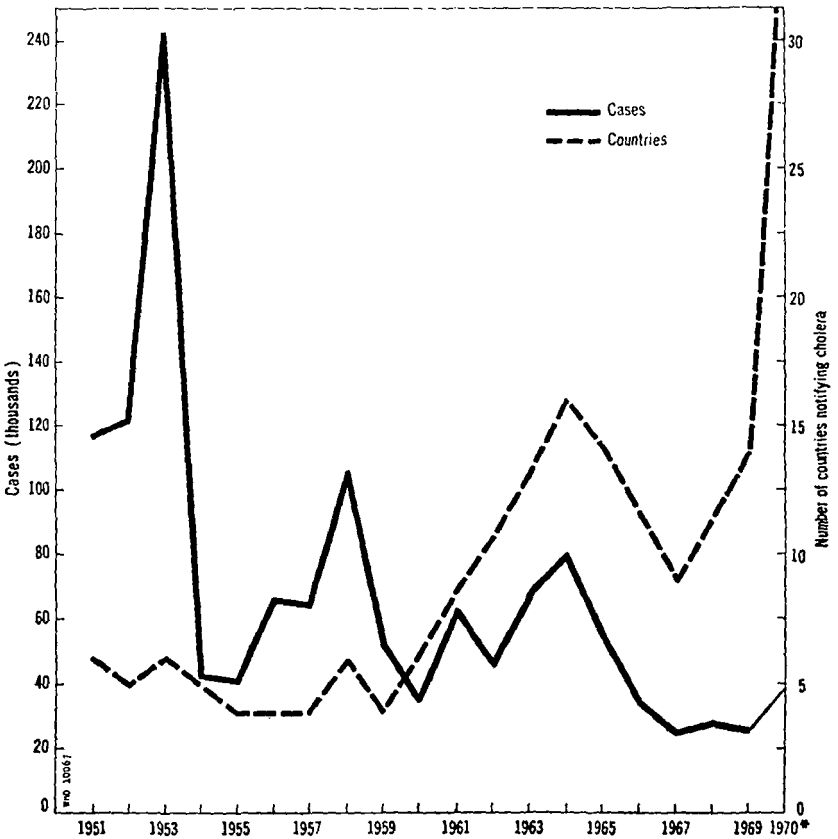


FIG. 2. INCIDENCE OF CHOLERA AND NUMBER OF COUNTRIES NOTIFYING CHOLERA, 1951-1970



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