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PROBLEMS OF CHOLERA CONTROL
IN THE EASTERN MEDITERRANEAN REGION
WITH SPECIAL REFERENCE TO EL TOR TYPE

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I FOREWORD

Cholera stands unique among the quarantinable diseases. It is the oldest known, and nowhere in the history of the pestilences that beset man was there a disease whose pandemic excursions were so widespread and so catastrophic in virulence.

Cholera stands unique also among the communicable diseases for, in spite of the discovery of its causative agent more than eight decades ago, yet there is still discrepant opinions among the different investigators about some of its attributive characteristics. The pathogenesis of the disease, the infectivity or safeness of the convalescent carrier, the degree of effective protection of vaccination, etc., are but a few of the aspects of the disease that need further study.

Yet, one must avow the fact that, to cholera, goes the credit of instigating, or rather initiating, international collaboration in the field of health. One might mention as well that, to cholera, also goes another credit of interest to this Region, and that is the institution of the first health administration in Egypt.

II HISTORY AND INCIDENCE

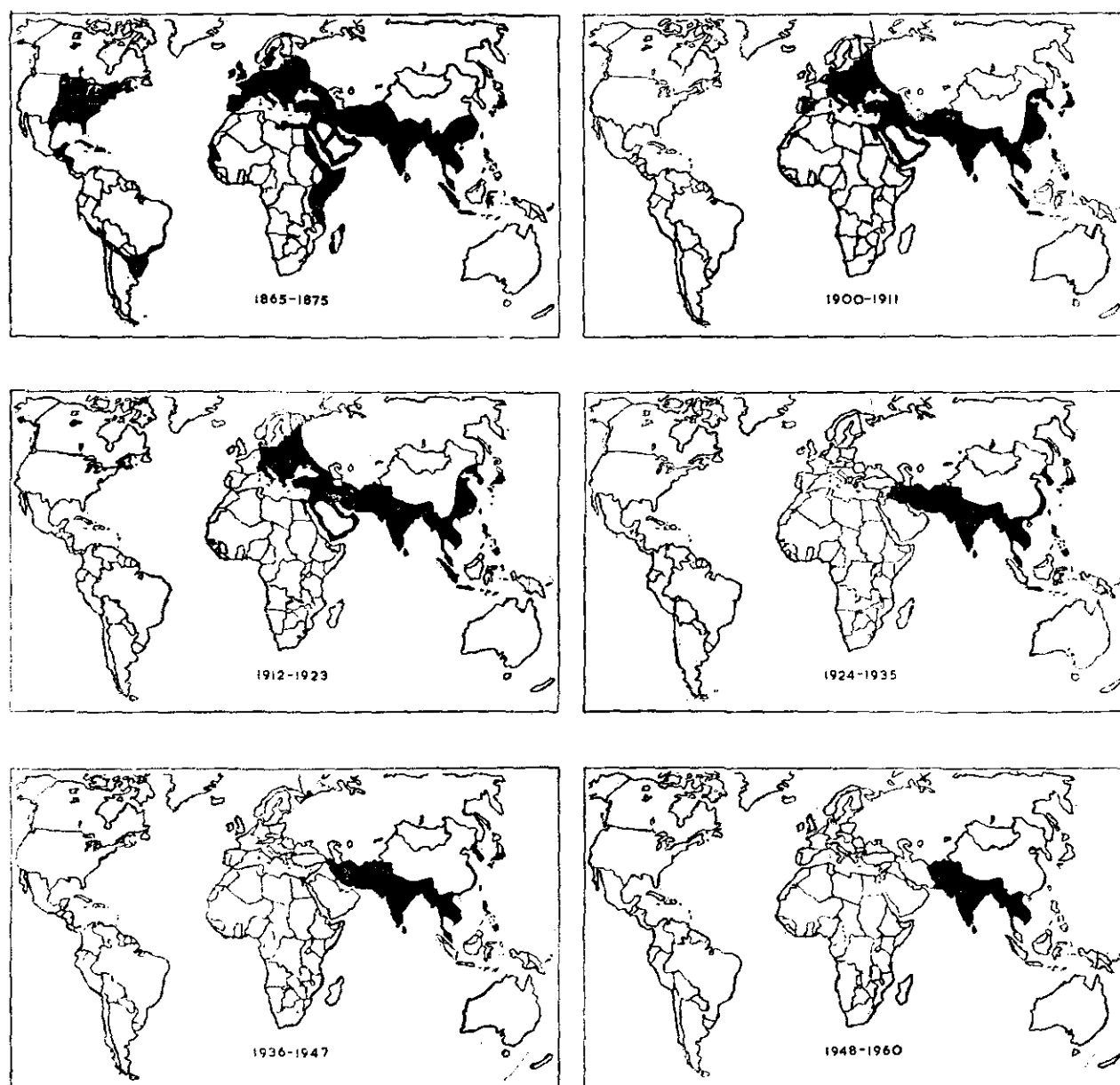
Although the history of cholera has shown its incidence has never ceased, yet one can safely divide its world incidence into four arbitrary periods:

First period: prior to 1817, "during which cholera was confined to the East, if not almost exclusively to India".

Second period: from 1817 to 1923, "during which pandemics originating from India spread to countries lying east and west of India and, in some cases, swept over several continents of the world".

Third period: from 1923 to 1960, during which "cholera became once more almost entirely a disease of the East, with the exception of an invasion of Iran and Irak in 1927 and 1931 and of Iran again in 1939". During this period two epidemics outside the Indian subcontinent were reported. The first in Egypt (1947) and the second in Syria 1948. They were not included in Pollitzer and Swaroop's third period, most probably because the theme of these authors' discussion was the role of the Indian subcontinent in disseminating infection, and as the origin of these two outbreaks had not been elucidated, they were excluded from their discussion.

FIG. 1 SPREAD OF CHOLERA IN THE WORLD
DURING 1865-1875 TO 1948-1960



Fourth period: from 1961 up to date, during which outbreaks of cholera caused by V. El Tor¹ have spread from Celebes and invaded nearly all the South-East Asian countries, India, Pakistan, Afghanistan, Thailand, and lately Iran, reaching northwards the USSR province contiguous to Afghanistan².

In contrast, "classic" cholera³ has not been reported outside India and Pakistan² during this period.

A. Cholera during the first three periods

Study of the available records of cholera during the first three periods delineated above, evinces three important epidemiological facts:

1. The disease occurring during all these epochs was of the "classic" kind - i.e. caused by V. Cholerae, the only exception recorded being the Celebes outbreaks (see later).
2. India and Pakistan³ are the habitats of the infection of cholera. All the records testify to its yearly occurrence in those two countries without interruption since records were kept. Not only this, but these same records also show that the combined share of cholera mortality of these two countries put together never fell in any decade or period below 93% of the total mortality of cholera all over the world, from 1901 till 1960, the last year of the first three periods under discussion. The average yearly toll of deaths caused by cholera for the period 1901-1960 reaches the figure of 243 309 deaths in India and Pakistan (pre-partition India). The corresponding figure for the rest of the world is 17 335 deaths.

These figures are for "classic" cholera.

Figure No.1 shows the spread of cholera in the world during 1865-1875 to 1948-1960 (reprinted from Kamal 1963).

¹As shall be mentioned later, it has been proved and acknowledged internationally that certain strains of V. El Tor could cause "cholera" indistinguishable clinically and epidemiologically from "cholera" caused by V. cholerae. This latter disease is always referred to as "classic" cholera.

²This is the official information as up to the time of writing this paper (first week of September 1965).

³Before 1947 Pakistan was part of India and together they are alluded to in the literature as pre-partition India.

3. Bengal¹ acts as the "fons et origo mali" of cholera, or to put it in the words of Pollitzer, "as the cradle, if not the original home of the infection". Proof of Bengal acting as the "fons" (home) evinces from the fact that it is responsible for a little less than a third of cholera mortality all over the world. The percentage for the years 1951-1960 was 31.24%. Swaroop in his study of endemicity of cholera in India has shown that at least eight of the districts situated in Bengal did not have even a single month which was free from cholera deaths during the 360 months studied.

The "origo mali" qualification conferred on Bengal is based on the results of the studies of both the pandemic and the epidemic spread of the disease since 1817, the year of the first reliable records of the incidence of cholera (Pollitzer 1959).

During the era of pandemic spread of cholera, all investigators agreed that the infection originated in pre-partition India. (Macnamara and others, quoted by Pollitzer). As regards the epidemic spread of the disease within the India subcontinent, the Report of the Central Expert Committee of the Indian Council of Medical Research (1959) earmarked Bengal as the focus whence infection disseminates, invading other states with the returning pilgrims, tradesmen, etc., at the finish of the religious rites (Figure 2).

Three important epidemiological facts can be deduced from this abridged exposition of the history of "classic" cholera.

1. Cholera is endemic in that part of Asia formerly known as pre-partition India and which now comprises India and Pakistan.
2. The "fons et origo mali" of the infection is Bengal. In other words, it is the area of the Ganges-Brahmaputra Delta that is responsible not only for keeping up the flame of endemicity, but also for the epidemic conflagrations with the potential danger of pandemic spread.
3. Movement of population from infected areas to clean ones, is an important means of spreading infection.

B. Cholera during the fourth period

This period is characterized by the persistence of classic cholera in the Bengal area and by a pandemic spread of El Tor cholera among the South East Pacific Region and a tendency of spread towards the West.

¹Bengal is the name of that area of land formed by the Ganges - Brahmaputra Delta. Although in the 1947 partition of India, Bengal was divided into West Bengal (which remained in India) and East Bengal, (named now as East Pakistan), yet from the epidemiological point of view, they must, as regards cholera, be considered as one area.

FIG. 2 PROBABLE ROUTES OF SPREAD OF CHOLERA - 1958



The number of classic cholera during this period is as follows (WHO Chron. 19/6, 1965):

<u>Year</u>	<u>Cases</u>	<u>Deaths</u>
1961	49 360	17 292
1962	28 183	10 039
1963	57 550 ¹	20 962 ¹
1964	81 400 ¹	19 571 ¹

As regards El Tor cholera, this will be discussed later on.

III CHOLERA AND THE COUNTRIES OF THE EASTERN MEDITERRANEAN REGION

Study of the six cholera pandemics show clearly the role pilgrimages played in the spread of the disease, invading neighbouring areas in sequence, both in order of geographical contiguity and chronologically. There is one exception to the above statement as to the role of pilgrimages. The first pandemic (1817-1823) was not related to pilgrimages; it originated through the landing of British troops sent from India to Oman. A point worth mentioning here is what Macnamara has concluded from his study of these pandemics; he found that, preceding every pandemic, a flare-up of the disease occurred in Bengal (in Pollitzer).

To show that the states covered by EMRO are so epidemiologically intimate that rarely one escapes cholera infection when its neighbour has already been invaded, the following chronology of the pandemic spread of cholera has been prepared (from Pollitzer):

First Pandemic 1817-1823

- 1820 Oman, Muscat
- 1821 Iran through Muscat
- 1821 Iraq (Basra, then Baghdad)
- 1822 Syria
- 1823 Iran for the second time, through the return of the Persian Army from Iraq

There is reference to cholera occurrence (1820-1821) in the Eastern coast of Africa through merchant boats arriving from Arabia. Whether Ethiopia and Somalia became involved in that pandemic or not is an open question.

¹These figures are from the Wkly Epid.Rec. (1965) 40, 16. Neither the table on page 202 nor the information on page 200 indicate clearly whether El Tor Cholera 1964 cases and deaths are included in these figures or not.

Second Pandemic 1829-1850

1829 Iran (via Afghanistan), Bahrein
1830 Arabia
1831 Egypt, Palestine, Syria and Tunisia
1834 Egypt
1835 Somalian coast, Ethiopia, Sudan
1835 Algiers for the second time, but this epidemic originated from France, and from Algiers it spread to Tunis
1837 Egypt
1846 Arabia, Iraq, Iran
1848 Egypt
1850 Egypt

Third Pandemic 1852-1863

Some believe that this pandemic was the combined result of local recrudescences and of repeated importations of the disease.

1853-1854 Iran
1855 Arabia, then to Syria, Egypt, Sudan, Tunis, Algiers
1859 Iran, Algiers (via France)

Fourth Pandemic 1863-1875

1865 Arabia, Egypt, Sudan, Ethiopia, Tunis, Algiers
1871 Egypt, Iran
1872 Sudan
1875 Syria

Fifth Pandemic 1881-1896

1882 Arabia, Tunis, Algiers
1883 Egypt
1892 Iran, Ethiopia
1895-1896 Egypt

Sixth Pandemic 1899-1923

1902 Arabia
1907 Arabia
1907-1908 Iran
1911-1912 Iran
1918 Palestine

This chronology leaves no doubt as to the potential danger to which the countries lying with EMRO are exposed whenever cholera embarks on an excursion north-westwards. That this danger is not imaginary finds proof in the present cholera El Tor spread¹. This epidemiological observation, or rather fact, deserves serious consideration by the public health administrators in this Region, especially as the infection has already invaded some states in the Region.

¹See later

IV VIBRIOS AND CHOLERA

A. Vibrio Cholerae

As far back as 1849, Snow and then Budd expressed their opinion that cholera is infectious. They even hypothesized that the disease has a causative agent. Snow called it a "morbid matter ... most likely ... a cell ... which passes from the sick to the healthy and which has the property of increasing and multiplying in the systems of the persons it attacks". Budd hypothesized the agent as "a distinct species of fungus which, being swallowed, becomes infinitely multiplied in the intestinal canal, and the action thus excited causes the flux of cholera, which with its consequences constitute the disease".

There is also mention in the literature that Leyden (1866) and Klob (1867) claimed their seeing vibrios in the dejecta of cholera patients, and Bruberger (1867) also claimed the same not only in the stools but also in the vomits (in Pollitzer).

Yet, the credit of finding and isolating the etiological vibrio of cholera goes to Koch. He did not only report their presence in the stools of patients but also in the intestines of the victims of the disease.

Consequent upon the universal acceptance of Koch's vibrio as the causative agent of cholera, extensive studies have been devoted to its laboratory identification, especially as morphologically similar vibrios were met with in the absence of cholera, especially those found in water.

Outside the morphological appearances of V. Cholerae, it was soon found that this organism has the following properties¹:

Grows well in alkaline media; forms a surface pellicle in broth; grows better under aerobic conditions than under anaerobic ones; grows well and rapidly in peptone water; multiplies rapidly in the presence of 0.5 to 2% sodium chloride; forms indole and gives nitroso-indole reaction (i.e. cholera red positive); does not form acetyl-methyl-carbinol (i.e. Voges Proskauer negative); forms acid but not gas from sucrose, maltose, mannose but no reaction with arbinose; non-haemolytic to sheep or goat blood; possesses a heat-stable O antigen; and shows dissociation from smooth to rough and rugose forms, etc.

Of course these characteristics took decades to be formulated and proven before they were accepted and adopted. The two tests which received more or

¹For detailed information on the properties of V. Cholerae, see Pollitzer, Chap.3, or Books on Bacteriology or Felsenfeld's WHO/Document PA/20.25, Geneva, 1965.

less general acknowledgement for differentiating V. Cholerae from other vibrios were its negative haemolytic reaction and its positive agglutination reaction with anti-O-serum.

Just as the recognition of V. Cholerae by using the above mentioned tests, with stress on the serological reaction, just as this appeared to be a perfectly straight forward matter, Gottslich (1905, 1906) suddenly threw a spanner into the works in the form of six strains of vibrios he isolated from the dead bodies of returned Mecca pilgrims at El Tor quarantine Station. These victims showed no signs of choleraic disease neither during life nor on postmortem examination. Gottslich also announced that applying the then known laboratory tests to these strains, they were found to comply positively with all the tests including agglutination with anti-O-serum. Soon it was found that Gottslich's strains were haemolytic in contrast to the non-haemolytic V. Cholerae, and they henceforth were designated as El Tor vibrios.

The interest and polemic which these strains excited, spurred further investigation of the causal organism of cholera, especially as shortly after the isolation of El Tor vibrios, it was found that they show precisely the same Inaba and Ogawa subtypes as V. Cholerae itself. With the continuance of controversies among the different workers about the relationship of V. El Tor Cholerae, it was noticed that different laboratories used different techniques and reagents, which might give different results. It was also noticed that vibrios have a tendency to dissociate, and tests with fresh isolated (smooth) organisms reacted, sometimes, differently to their rough variants, hence the different reports.

Persuance of study led to rejecting the suggestion that the haemolytic V. El Tor constituted any menace to man, and to continue recognizing V. Cholerae (of Koch) as the sole etiological agent of cholera. Though this decision has met international approval and adoption, yet it found dissenters. However, international quarantine and the disease statistics were based on the concept of V. Cholerae being the sole pathogen of cholera.

B. Vibrio El Tor

In September 1937, V. El Tor threw another spanner which shook, rather stronglier than before, the proponents of the soleness of V. Cholerae as the etiological agent of cholera disease. During that month, cases of "cholera-like" disease occurred in South-West Celebes (Sulawesi) in Indonesia. The

outbreak continued for eight months with forty-eight cases. The features of that outbreak, as recorded by de Moor (1948-1949), were: disease clinically identical with classic cholera; high case fatality rate (70%); disease occurred as one case per house; cases sporadic with no tendency to spread epidemically, a deduction reached at by de Moor because the forty-eight cases occurred in a coastal area extending over a length of about 100 km; the organism isolated from the cases was V. El Tor. Between October 1939 and April 1940, another outbreak affecting sixty-five persons was observed. This second outbreak showed the same features clinically, epidemiologically and bacteriologically as its predecessor. During the gap between the two outbreaks, the area was free from the disease.

These incidences raised again the question of the pathogenicity of V. El Tor. But the exclusive localization of the disease in South Celebes, the more or less sporadic character of the cases, the non-recurrence of the disease within short intervals etc., made de Moor hypothesize "occasional pathogenicity" of V. El Tor in contradistinction to the "dynamic pathogenicity" of V. Cholerae. And on such a hypothesis, de Moor described V. El Tor infections as "static" in character without any tendency to spread epidemically.

de Moor's opinion was accepted and the Celebes disease was called "paracholera", and needed no international notification of its occurrence.

The entanglement of this part of the world in World War II activities and its occupation by the Japanese prevented further information.

But, beginning January 1957, a series of outbreaks of "paracholera El Tor" broke out in that territory. Thus seventy cases occurred in Makassar town between January 1957 and February 1958, and eighty-one cases were reported in the adjacent areas. Two cases occurred in Djakarta, in the island of Java. There was again a gap which was followed by outbreaks in which the disease did not only attack several islands and sections of Indonesia, but spread to most of the islands of the Western Pacific and also to many countries in South and South-East Asia¹. This pandemic spread of a disease non-differentiable from classic cholera in all the latter's characteristics, did put the health authorities of all countries in a non-enviable position. The International Sanitary Regulations prohibited taking any anti-measures because "paracholera" had not been acknowledged as a quarantinable disease.

¹See later for details.

This prompted the World Health Organization to convene a "Study Group" to study the problem. They decided that El Tor infection should be regarded as essentially identical with classical cholera and treated as such (1962). That decision was approved by the Committee on International Quarantine. And the Fifteenth World Health Assembly, in 1962, accepted the proposal of the above-mentioned Committee that cholera should be defined in the International Sanitary Regulations as including Cholera El Tor.

C. Non-Agglutinable Vibrios (NAG)

It has long been observed and reported that, in not a few outbreaks of cholera-like disease, only NAG vibrios could be isolated. Investigators of such outbreaks resorted to a diagnosis of "gastroenteritis", in spite of their conviction that the outbreaks were cholera from both the symptomatological and epidemiological aspects. Some other investigators recorded that, during inter-epidemic periods, only NAG vibrios could be isolated from cases of clinical cholera while during the epidemic stage only agglutinable vibrios (AGs) were isolated; others still recorded isolation of NAG vibrios along with V. cholera from the stools of cholera patients.

These observations, added to the presence of the NAG vibrios in surface water, have led some to doubt their pathogenicity.

Dutta et al (1963) have examined this question and reported that they were able to produce cholera-like disease in the infant rabbit resembling human cholera. The "infant rabbits infected with NAG suffered from profuse diarrhoea (rice water stool), dehydration, anuria, etc. The post-mortem picture, too, resembled that of an attack due to Ogawa or Inaba". The same authors reported that "some of the cholera-like vibrios isolated from water sources could, under favourable circumstances, produce cholera-like disease in the infant rabbit".

Ramanarayan et al (1963) reported on an outbreak of cholera that occurred in Guntur and Kirshna districts of Andhra Pradesh State in India. The results of stool examination of 172 cases were as follows: 143 were positive for V. Cholerae, (133 Ogawa, 8 Inaba, and 2 Hikojhema); from the stools of the rest (twenty-nine patients) only NAG vibrios could be isolated.

McIntyre et al (1965), studying an outbreak of diarrhoea, found that the sole pathogen isolated from the stools of cases was what they termed "non-cholera vibrio", NCV, a term introduced by the authors as a synonym to NAG. These authors took advantage of the infant rabbit being a model for the experimental

reproduction of cholera-like disease and were successful in completely protecting the animals from the consequences of infection with the isolated NCV by the administration of hyper-immune serum. Resorting to serology, the authors found unequivocal antibody titre rises against the infecting NCV in the sera from certain (but not all) cases. A survey in the area failed to reveal any people with heavy infection of NCV (NAGs).

Bhattacharji and Bose (not published yet) delivered a report during the Seminar on Cholera organized by the Indian Council of Medical Research in New Delhi in 1963. These two authors report that they have been successful in changing Inaba cholera vibrios to the NAG forms within 24 hours of their addition to vibrio free samples of natural waters; moreover they report that they have been able to convert these NAG forms to the original AG vibrios or the human pathogenic variety, by passaging in the laboratory these inagglutinable vibrios of Heiberg types I to III, through suckling rabbits of not above ten days of age and infected by intraoral feeding. They add that the cholera vibrios, after each passage in water or rabbits, could then be isolated in pure culture.

From the results of their experiments, they hypothesize that the NAG Cholera vibrios are only a rough phase of the AG vibrio and a part of the life cycle of a *V. Cholerae*, and it is in this NAG form that cholera infection probably persists in the environment. When such NAG vibrios are ingested by man, then depending upon their Heiberg type and whether the pH of the gut in the host is favourable for infection to take place or not, they become liable to get mutated into the agglutinable or the pathogenic stage, or at times, if belonging to NAG types I to III, they themselves could cause infection. Thus the authors contend that this AG-NAG is the cycle of development of *V. Cholerae* in nature, and that cholera infection in the environment probably persists in the form of these NAG Vibrios. Such opinions must wait confirmation by further study.

Nevertheless, there is not much opposition to the fact that occasionally NAG vibrios could produce cholera-like disease. Felsenfeld (1965) states "There has been a tendency in some quarters to deny the pathogenicity of NAG vibrios. This may be a fallacy. The O antigen of cholera vibrios is not necessarily identical with the choleraemic toxicity. There is no reason why some non-agglutinating vibrios could not harbour or develop a choleraemic principle. It is, therefore, desirable to pay more attention to such organisms than they were afforded in the past".

V THE PRESENT PANDEMIC OF CHOLERA EL TOR

When discussing the history of cholera, it was mentioned that our knowledge of the occurrence of this disease could be discussed in four arbitrary periods. The first three ended in 1960 and the fourth began with 1961 and we still live with it.

This last period could rightly be called the period of the first pandemic of cholera El Tor.

The preceding pages tell the story of V. El Tor, its first isolation from humans and how it lost its case as a pathogen of cholera disease. However, it did not take long to prove itself equal to V. Cholerae not only as a cholera disease pathogen, but equal to it also in two other attributes: epidemicity and pandemicity.

A. Course of the Pandemic

In January 1961¹, the disease flared up in Sulawesi and from there started the pandemic excursion with which the world is confronted at the present time. In May of the same year, the disease began to appear in Java and Sumatra. de Moor (1963) mentions that, from October 1961 till April 1962, 1819 cases with 270 deaths were recorded in Java, in Sumatra 251 cases with 48 deaths, and in the southern part of Celebes 148 cases with 72 deaths.

Sarawak became infected in July 1961 and until October there were reported 582 cases with seventy-nine deaths.

Infection reached Hong Kong and Macao in August 1961, and within two weeks seventy-six cases with fifteen deaths were reported from Hong Kong. In Macao, fifteen cases with five deaths were reported.

During June and July 1961, rumours were circulating about a cholera epidemic in Kwantung. It was broadcasted as paracholera with 234 cases.

Around the end of September 1961, the Phillipines was invaded, and Luzon became implicated. From September 1961 to the end of the year, 6061 cases with 771 deaths were recorded in the Phillipines and during the first three months of 1962, there were 3 860 cases with 561 deaths. The disease continued to occur in that country at the rate of twelve cases per week (de Moor).

¹The information about the geographical spread of the pandemic, its dates of appearance, its incidences etc. are abstracted from many sources, mainly de Moor (1963), Felsenfeld (1963) and Mukerjee (1964) etc. They must be looked at as approximate.

North Borneo was infected in January 1962, and in July of the same year Formosa began to have its share of the disease. Within three months there were 382 cases with twenty-four deaths in that State.

Hong Kong and Macao reported new cases during August and October, and in November Sarawak appeared with fifty-six cases and nineteen deaths.

On 10 October 1962, Irian Barat (New West Guinea) became infected and 1364 cases with 485 deaths were recorded.

Taiwan (China) reported 383 cases and twenty-four deaths in 1962.

In 1963, Cholera El Tor appeared in Malaya and Thailand, then Burma and East Pakistan. According to Barua *et al* (1964) the first case of El Tor Cholera detected in India (Calcutta) was on 1 April 1964, and by the middle of May of the same year cases of El Tor Cholera were already distributed over scattered areas in Calcutta and had already crossed to Howrah, which is situated on the other bank of River Hooghly. By October of the same year, V. El Tor became the more predominating type in Calcutta (Barna and Mukerjee 1964).

Spread westward was noticed and the disease reached Bombay. The line of further spread was towards the north, Afghanistan (July 1965) then Iran (August), Bahrein (August) and USSR (August also).

In Bahrein one case only was reported and the territory was declared free of the disease on 21 August 1965.

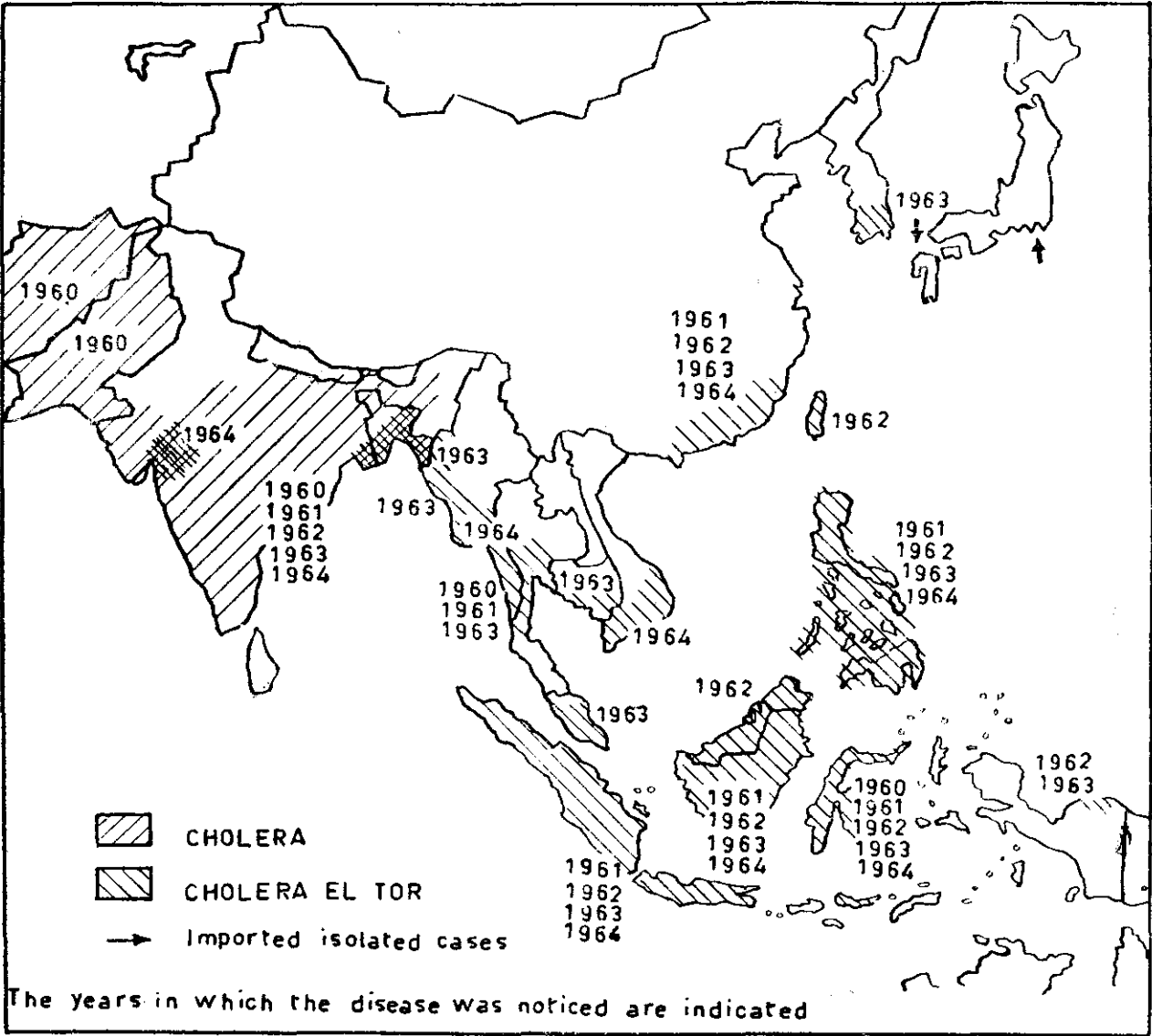
The following table gives the recorded incidence and mortality from Cholera El Tor during 1965 up to the week ending 28 August. Figure 3 shows the distribution of classic cholera and cholera El Tor since 1960¹.

NUMBER OF CASES AND DEATHS FROM CHOLERA EL TOR
REPORTED IN 1965 (1 January - 28 August)

Country	Cases	Deaths
Afghanistan	208	53
Bahrein	1	0
Iran	2 539	346
Malaya	1	0
Nepal	717	97
Philippines	474	128
Thailand	52	3
USSR (Uzbekistan SSR)	present	
Vietnam	900	18

¹The figure is reproduced from WHO Chronicle (1965), 19, 6

FIG. 3 DISTRIBUTION OF CHOLERA AND CHOLERA EL TOR SINCE 1960



The following table gives the world incidence (in cases and deaths) of cholera El Tor since January 1961.

Year	Cases	Deaths
1961	12 096	1 969
1962	13 392	1 969
1963 ¹	7 545	763
1964 ¹	37 384	2 516
1965 till week ending 28 August	4 892	645

These figures give a case fatality rate of 13.18%

B. Man and Pandemic Spread

One of the attributes of cholera disease, or rather its most outstanding one, is "individuality". By this is meant that cholera has, all these centuries, shown no modification or variation in its symptomatology, its pathology or in its epidemiology².

Individuality in the epidemiologic behaviour of cholera needs no other evidence than the role man himself plays in its spread, both epidemically and pandemically. Study of these two kinds of spread of cholera whether old or recent, has earmarked man, or to be more specific man's body, as the most important vehicle of transport of infection from an infected area to one free of infection, whether these two areas are within the same country or belong to different ones. The chronological appearance of the disease in the different countries during pandemics, when these countries are contiguous is proof enough. The time that lapses before infection makes its appearance in the new locality depends on the mode of travel, whether by road, train, ship or by air. History has shown that cholera never extends from place to place faster than man can travel.

The present pandemic of cholera El Tor is no exception, neither were its epidemic spreads within the different countries.

¹The figures for this year include suspected: cases 16 360 - deaths 705, in Vietnam Republic.

²One might venture and add its bacteriology too, because in no other specific agent of a communicable disease was there a relatively high degree of uncertainty about the significance of the many variants, the agglutinability, the haemolytic property of the vibrios and the relationship of all this to pathogenicity.

When cholera broke up in Sulawesi during 1937-1940, de Moor - observing the sporadic occurrence of the disease over relatively wide areas - hypothesized the "static" property of V. El Tor. Felsenfeld (1962 - personal communication and 1963) who studied the problem by touring the South-east Asian territories, explains that sporadicity by the circumstances that prevailed in the area during that period of time, and which restricted movement of the population. When conditions however permitted freedom of movement, the disease spread from Sulawesi to other areas of Celebes.

This local increase, not only in the number of cases but also in the number of infected foci became the spark that ignited the pandemic. It has to be remembered also that there is a big activity of sea traffic between the different countries in that part of Asia. And as Felsenfeld puts it "~~there is also a~~ certain amount of illegal movement of goods and displaced persons between those countries which makes epidemiological surveillance even more difficult than it is per se in countries consisting of many islands".

The different reports issued about the first cases isolated in the different countries show that they occurred either among members of fishing communities, or among families residing in boats or junks, or in the slum quarters found near the sites where ships from many neighbouring countries call. The disease was imported into Hong Kong (MacKenzie 1961) and into Manila in the Phillipines (Felsenfeld 1963; Wallace et al 1964) in this way. Sarawak was infected in a similar fashion. "During the last days of June 1961 a regatta took place in Kuching, Sarawak, in which boats from Celebes participated. The first cholera cases appeared on 1 July in that part of Kuching, called Surabaya, and where the boats were anchored" (Felsenfeld 1963).

Mukerjee (1964) in discussing how cholera El Tor reached Calcutta in April 1964, mentions that it was assumed at first that the refugees immigrating from East Pakistan to Calcutta carried the infection with them as it was known that cholera El Tor have been present in East Pakistan towards the end of 1963. This assumption was based on precedents and experience with the exchange of classic cholera between East Pakistan and West Bengal. But the testing of eighteen strains of El Tor vibrios from the former country proved them to belong to a phage-type (see later) different from those of the Calcutta strains. And on the basis of this, Mukerjee is of the opinion that El Tor infection may have spread to India by the sea route from Burma or Thailand.

The spread of El Tor infection from Calcutta to West India, West Pakistan and thence to Afghanistan and Iran needs no comment as no doubt the infection followed the customary pathway it tracked before, trampling human lives as it proceeds.

VI CHOLERA, A MEDICAL EMERGENCY

Cholera must never be dealt with as that pathologic condition conventionally termed disease. Cholera must be dealt with as an EMERGENCY that necessitates immediate action.

History and experience have decreed this action in the following two words: SUSPECT and NOTIFY.

Suspicion makes immediate treatment mandatory to save the life of the patient. Cholera patients treated within six hours of the onset of the disease rarely die.

Notification of the case to the medical officer of health saves the community from the catastrophic havoc that ensues when it steps in the shoes of an epidemic, which, it is the duty of the MOH to prevent.

The collaboration of the attending physician with the health authorities is essential from the start.

A. Symptomatology

Details of this are en-dehors this document¹, and it is only the first symptoms which lead to "suspicion" that are going to be tackled.

Clinical cholera is a bacterial enteric infection, characterized in its typical form by an unrelenting purge leading to dehydration which knows no peer in clinical medicine, and which sometimes is so acutely rapid as to out-distance treatment. The word clinical is purposely affixed to differentiate the typical, or as sometimes called the classical, forms of the disease from those milder forms which though choleraic in aetiology, yet the illness might be relatively less acute as to elude diagnosis by the attending physician, especially if the typical rice-water stools have not yet developed. The diarrhoea is accompanied by vomiting and soon dehydration becomes manifest as a result of the copious purging and vomiting.

The most common and ready diagnosis of an acute syndrome of vomiting and diarrhoea is "food poisoning", or "gastro-enteritis" if the patient is young. If such a diagnosis is admissible in ordinary times, it must be understood that it is not justifiable under the present circumstances of the danger of entry of cholera infection. This patient might be the clue to the presence of cholera.

¹See Pollitzer and textbooks of Tropical Medicine

The following incident, taken from the files of the 1947 cholera epidemic in Egypt, is a good illustration. Carrying out an investigation (Kamal - unpublished data) to find out the source (and mode) of the infection, two physician-certified-deaths from "food poisoning" were found registered in the official death registers of two of the villages of Sharkia Province (the new name for the "province" is governorate). These villages were situated south of Korein on the Ismailia sweet water canal, a few kilometres apart. The middle age of the deceased, the non-relationship between them and the affirmation of their relatives that nobody among the households complained of the same symptoms at the time (vomiting and diarrhoea) raised suspicion about the inaccuracy of the diagnosis. Discussing the matter with the physician who attended the two victims before death, he admitted that a retrospective diagnosis of cholera will be more in line with the disease picture he saw. These two patients were attended to medically between seven to nine days before the first batch of suspicious deaths occurred in Korein - a period of time the health administration lost, and a period of time sufficient enough for the infection to spread.

Now that all countries of the region are threatened by the invasion of this deadly disease, it becomes pertinent that their health administrations alert all members of the medical profession on the subject, in the manner they find fit, so as to spot any focus of infection at the appropriate time.

Although both cholera and toxigenic staphylococcus food poisoning declare their onset by vomiting and diarrhoea, yet critical scrutiny to some details regarding these two symptoms helps. Thus the vomiting in food poisoning always precedes the diarrhoea, is accompanied by nausea, retching and painful discomfort in the epigastric region. Also after a few vomits, the output will be small in amount. In cholera the vomiting in most cases comes after the diarrhoea, it is voluminous, continuous and painless.

As regards diarrhoea, in food-poisoning, it is usually preceded by colic, but not in cholera. In the latter disease, the purging happens without the patient being able to prevent it; in food poisoning, the patient is still strong enough to hurry to the latrine.

Relation in time to the partaking of food could usually be asserted in food poisoning, while in cholera there is no such relationship. If the food

has been shared by the household or by a group, other cases of food poisoning are found. In cholera, multiple cases in a household is a rare, or at least a highly infrequent, occurrence (see later).

Another differentiating point is that, in staphylococcal food poisoning, the symptoms usually abate in a few hours, while in cholera the patient's condition gets worse. Thus one of the means that could help in reaching a provisional diagnosis, pending bacteriological verification, is the isolation of the patient in hospital for observation (and necessary treatment).

B. Treatment

Historically, the treatment of cholera could be divided into two era, before and after the 1947 epidemic in Egypt. The research done on cholera patients during that epidemic has revolutionized the treatment of this disease, consequent on the biochemical studies of the disease. These studies have come up with the following facts.

1. The diarrhoea of cholera constitutes the entire pathologic aspects of the disease, and all the derangements of function seen can be accounted for on this basis.
2. The dehydration is isotonic with excessive losses of K^+ and HCO_3^- .
3. The cholera stool is no transudate as it contains about 0.1 g% protein, while a transudate contains about 2 g% of protein.
4. The ideal therapy consists of rehydration, i.e. intravenous replacement of the water and electrolytes lost in the stools and vomitus, and correction of the acidosis.

On the basis of these studies¹, a therapeutic regimen was developed and used. This regimen has proved its efficacy when applied in Bangkok, Hong Kong, the Philippines etc. when cholera broke out in those territories.

The regimen is based on knowing the degree of dehydration. This is easily estimated by determining the specific gravity of plasma and whole blood, by the copper sulphate method (Phillips et al 1950).

It is recommended that the degree of dehydration be estimated by the specific gravity of the plasma, rather than of whole blood, since cholera patients are usually anaemic. But whole blood specific gravity could be used when lack of a centrifuge and anticoagulants makes the determination of plasma specific gravity impossible.

¹These studies were carried out by NAMRU-3 Staff in Cairo. The authors are Weaver et al 1948 and Johnson et al 1948.

For each 0.001 elevation of plasma specific gravity above 1.025, 200 ml of isotonic saline are infused very rapidly. If whole blood specific gravity is the one estimated, 150 ml of isotonic saline is administered in the same manner for every 0.001 increase in the specific gravity above 1.055.

This amount of saline should be infused rapidly (up to 100 ml per minute). When this volume of solution has been given the infusion should be continued but at a slower rate (3 - 10 ml per minute).

As most cholera patients may continue to lose large volumes of fluids for two or more days, therefore maintenance of hydration by replacement of the lost fluid has to continue. The easiest method (in case measurement of the faeces, urine and vomitus lost is not practical) is by frequent determinations of plasma or whole blood specific gravity.

These determinations, ideally, have to be done every two hours, but in an epidemic situation with large numbers of patients, they may be made less frequently (every 4 - 6 hours). However, in cases with suppression of urine the two hourly determination is imperative. Maintenance hydration can be carried out according to whole blood specific gravity estimations giving 150 - 200 ml of isotonic saline for every 0.001 elevation above the normal¹.

Insensible fluid loss (in sweat, expired air etc) has to be considered. It amounts to about 1 200 ml per 24 hours. This has to be replaced by adding an equal volume of the maintenance fluid to the infusion.

Adequate hydration is usually reached when the volume of urine amounts to 60 ml per hour.

Acidosis is likely to occur in acute cholera since the stools contain on the average 15 m Eq. of bicarbonate per litre. Its prompt correction is pertinent.

In the absence of facilities for estimating alkaline reserve, a useful rule of thumb is to give 500 ml of 2% sodium bicarbonate solution for every four litres of saline.

~~Hypokalaemia often~~ complicates severe cholera, since the stools may contain 16 m Eq K^+ per litre. Adequate replacement of this ion is attained by adding 10 m Eq (5 ml of a 15% solution of KCl) to each litre of infused saline solution. Oral potassium (1-2 mg daily) should be given during convalescence.

¹This normal differs from country to country. During the 1947 epidemic in Egypt a whole blood specific gravity of 1.054 was considered normal.

Since the blood in untreated cholera patients is inevitably hypertonic, there is no justification for the administration of hypertonic saline solution or plasma. One should rely exclusively on normal saline, 2% sodium bicarbonate and 15% potassium chloride solutions.

Caution is enjoined in the use of isotonic saline and 2% bicarbonate infusions in infants and small children, and in those with renal and cardiac disease. In them, additional every two-hour supervision by repeated plasma specific gravity estimations and by measurement of total fluid output is required in order to avoid hypervolaemia leading to pulmonary oedema. When renal damage is shown by oliguria, sensible and insensible fluid loss only should be replaced by (5%) solution of glucose in water. (Morgan et al 1959).

Phillips et al (1963a) in their study of the gastro-intestinal physiology in cholera commented on prescribing fluids orally in the therapy of cholera, whether as simple water or as solution of electrolytes; in their opinion there is no salutary effect to recommend this procedure. They even warn against giving water ad libitum, two glasses of water an hour, but not more

Antibiotic Therapy

During the 1947 epidemic, sulphaguanidine with sulphadiazine, were given a trial (Kamal et al 1948). The results were not conclusive except that the drugs speeded the clearance of the stools from the vibrios.

Antibiotics have been tried by several workers who reported favourably on their use. Chandhuri et al (1950) using chloromycetin per os reported rapid disappearance of V. Cholerae from the stools and slightly earlier appearance of formed stools. Das et al (ibid) obtained similar results with terramycin. Morgan et al (1959) however failed to find any beneficial effect from antibiotics. Carpenter et al (1964) carried out a controlled study on two groups of patients, each group included ten. One was given tetracycline and the other left as a control. The regimen of treatment was: 100 mg intravenously on admission to be repeated 6-hourly for the first 24 hours; 500 mg orally two hours after admission and 500 mg orally 6-hourly for the first three hospital days. All patients in the two groups received intravenous isotonic saline and alkali therapy in the ratio of 2: 1 and no other medication. The organisms disappeared in the tetracycline group within 24 hours in contrast to an average of six days in the other group. The other point these workers raise is that the volume of stools

for the antibiotic group diminished after the first 24 hours to approximately half the volume of the stool of the control. The stool in the antibiotic group becomes formed earlier too and thus cholera patients treated with this antibiotic could be discharged within three days with a formed vibrio free stool¹

VII PATHOGENESIS

The full understanding of the pathogenesis of cholera has been delayed for long. There are two important reasons for that delay:

1. Research workers tried to get an understanding of the pathogenesis of the disease by trying to reproduce the disease in animals. The inability to reproduce it in experimental animals with assured regularity has greatly hampered reaching a definite opinion.
2. Research workers gave the clinical, bacteriological and epidemiological aspects of the disease all their interest and efforts, disregarding the role of the physiologist.

The theories and opinions advanced to elucidate the intricacies of the pathogenesis of cholera are many, varied, and some of them controversial and/or needing verification.

The two theories which are based on laboratory and/or field evidence are:

1. The endotoxin theory.
2. The "sodium pump" inhibition theory.

A. The Endotoxin Theory

Many workers claimed that they were able to prove the production of an exotoxin or an endotoxin from *V. Cholerae* by applying certain laboratory techniques. When these procedures were repeated by other workers the result were discordant.

But, with the infant rabbit technique introduced by Dutta and Habbu (1955), encouraging results were reached.

Dutta and Habbu, followed by others, have reported their success in producing in infant (10-day old) rabbits a disease which closely resembled human cholera. Their technique was inoculating those animals intra-intestinally by a small inoculum of *V. Cholerae*. Diarrhoea developed

¹The results reached at by this study are a very important contribution to the therapy of cholera. What gives it this importance is the authors' gauging of the efficacy of the treatment by measurement of the fluid loss.

within 20 to 24 hours, and invariably the animals died in another 10 hours as a result of dehydration. When sonic disintegrated vibrios were administered orally to those animals, the same disease picture ~~was~~ produced, but death ensued more quickly. These experiments pointed to an endotoxin, but when such a principle was prepared and administered to infant rabbits no diarrhoea developed although the animals died.

Finkelstein et al (1963) tested 46 sterile filtrates of stools from patients with clinical cholera for antigen activity and for toxicity for 11-day chick embryos. They found that only those filtrates with high antigen activity were toxic to chick embryos. They conclude that the "results indicate that a considerable amount of endotoxin is potentially available in the gut of cholera patients to contribute to the pathogenesis but its relationship to the disease has not been demonstrated".

The important findings in both experiments are that the animals suffer from profuse diarrhoea, extreme dehydration and haemo-concentration. In both also, the epithelial lining of the intestine remained intact. This last finding is in line with Gangrosa's work on humans.

B. The "Sodium-pump" Inhibition Theory

The impetus to theorizing this to explain the dehydration began with the finding that the stools of cholera patients contains about 0.1 g% of protein. At that time, the trend of thought was that the big volumes of fluid passed by the cholera patient in his stool is a transudate consequent on denudation of the intestinal mucosa. But as a transudate contains 2 g% of protein, this was considered as a strong evidence against the denudation hypothesis (Weaver et al 1948).

Support for this came in 1959 when Gangrosa et al (1960) failed to observe, through histological examination, any loss of mucosal continuity in the snippets of intestinal mucosa obtained by Crosby capsules from cholera patients.

Later Gordon "developed an elegant method for quantitating protein loss from plasma through the intestine into the bowel lumen. He tagged polyvinyl pyrrolidone (PVP) with Iodine - 131, PVP was chosen because the material was not attacked by intestinal bacteria. He found that when his iodine-131-tagged PVP was infused intravenously into cholera patients, there was no greater PVP

content in the stool of cholera patients per unit time than there was in the stools of normal individuals (in Phillips 1963).

Thus the denundation theory dropped into oblivion for ever, giving birth to the "sodium-pump" theory (Phillips 1963). This theory is based on the fact that normally, in a healthy person, there is continuous movement of large volumes of electrolyte solutions from plasma to gut lumen and from there to plasma, resulting in a net fluid flux of zero.

There is evidence to show that, in man, the volume of fluids passing from blood to gut lumen and vice versa, runs to about 40-80 litres in a man of 50 kilogrammes. Further, it has been proved "that this flux of fluid from the gut lumen to plasma is accomplished by an active transport of sodium". In cholera this sodium pump is inhibited, thus causing that big volume of stools (Phillips 1963). Later, it was possible to prove the presence of a sodium-pump inhibitor in the stool water of cholera patients, but not in the stool water of normal health individuals. This finding is consistent and there was no difference between the sodium-pump inhibitor obtained from stools of patients with classic cholera or from stools of cholera El Tor patients.

Barua and Sack (1963) found C - reactive protein in the sera of almost all the cholera patients usually within 24-48 hours of onset. The same was noticed in non-cholera diarrhoea cases. In the authors' opinion, this finding favours the view that the clinical syndrome of cholera is associated with acute inflammation of the intestine rather than specific interference in fluid transport across the gut wall only.

Rainsford (1952) suggested that cholera is an allergic or atopic reaction to the presence of the vibrio. Panse and Dutta (1963) gave evidence that cholera toxin is capable of releasing histamine from the tissues. In their opinion the shock-like symptoms in clinical cholera could be explained on the basis of histamine released in the circulation by the cholera toxin.

The final word on the pathogenesis of cholera has not been said yet.

VIII DIAGNOSIS

Cholera can be diagnosed on clinical grounds by bacteriological tests and it could also be diagnosed on epidemiological evidence.

The classic form of the disease is so typical in its symptomatology that little difficulty is met with in its recognition, especially when the

attending physician has seen a case or more before. The difficulty arises only when the case is the first one met with in the locality, especially when the locality is free from the disease. But there are certain circumstances that could help, or rather lead, him to suspect cholera. For example if the patient is a new-comer from an infected area, or his illness has been preceded by deaths in the family or in the locality after an attack of a similar disease.

This last circumstance was the only lead to the discovery of the disease in 1947 in Egypt. The MOH of Korein noticed multiple deaths in his village for three consecutive days. The symptoms complained of by the deceased were acute diarrhoea and vomiting. As enquiry showed that there was no fair, festival, or social gathering with communal feeding, e.g. marriages, and as there was no public water supply in the village, shigellosis and food poisoning were ruled out and cholera was suspected, a decision which proved to be wise.

Laboratory Diagnosis

The aim of resorting to the laboratory is verification of the presumptive diagnosis. The steps in laboratory testing in cholera are:

- a. sampling
- b. culturing
- c. isolation and identification of the vibrios.

A. Sampling - Culturing

Vibrios leave the body of patients in the stool and in the vomit. A sample of both should be taken especially when dealing with the first few cases.

Lately, rectal swabs have been introduced to simplify matters. Specimens of the stool itself are imperative for the first few cases in a locality, as isolation of the vibrios is more guaranteed. Rectal swabs are practical for sampling convalescents, contacts and groups of populations. Dry swabs are useless, they must be wetted with one of the preserving media as Venkatraman and Ramakrishnan's alkaline sea-salt water, or alkaline peptone water, to which is added potassium tellurite, before taking the specimen.

The tube into which the swab is put must contain also an amount of the preserving medium enough to keep the end of the swab immersed in it. Dried-out stools are also useless. When sampling fluid stool, it must be put in a receptacle containing at least 10 ml of the preserving fluid and then shaken thoroughly.

Felsenfeld (1965) states that alkaline peptone water is most favourable for short-term preservation - not more than one day. He also advises incubating it for 6-8 hours upon arrival in the laboratory before plating. Alkaline sea-salt is not usually incubated.

These details have been mentioned intentionally because carelessness in abiding with them deleteriously affects the results of the tests carried out.

Different countries have different regulations about the way cholera specimens are sent to the laboratory. In Egypt they have to be delivered by a messenger and sending them by mail is taboo.

The procedure of culturing differs with different workers. details of such procedures are outside the scope of this document.

At the end of incubation, the bacteriologist has to pick up the suspicious colonies for study. The necessity to study as many colonies as possible cannot be overemphasized because of the possibility of simultaneous infections with several vibrio strains, and the appearance of variant and mutual organisms. (Felsenfeld ibid).

B. Identification and Differentiation

The final step is the identification of the isolated vibrio.

Agglutinability and haemolysis as differentiating characteristics between the different vibrios have lately lost their time-honoured specificity.

Although research in vibrios continued since Koch's isolation of *V. Cholerae*, yet it was the acknowledgement of *V. El Tor* as the causative agent in the pandemo-epidemic spread of cholera in the South Pacific, that instigated research on a rather bigger scale. And no doubt that this latter vibrio surprised the scientific world not only by its new attributes, but also by its capability to variate some of its known characteristics. An example of the variation is the scum formation the different strains show when grown in meat extract or meat infusion broth. Anaerobically grown, they form no scum. Another example, and a better one, is its haemolytic property. Some of the strains are stably haemolytic, others are stably non-haemolytic and a third group was noticed consisting of strains dissociating from apparently non-haemolytic to haemolytic form. Some of the haemolytic strains may lose their haemolytic ability while a non-haemolytic one may acquire the haemolytic property. These

alterations in the haemolytic character occur in an irregular fashion to an extent that makes it pertinent to conclude that, in El Tor strains, haemolysis is so variable a character that its use for differentiating it from V. Cholerae is not always adequate for the purpose. (Mukerjee and Roy, 1962; Roy and Mukerjee, 1963; Barua and Mukerjee, 1964 and Felsenfeld, 1964).

Differentiation of the Isolated Vibrio

The pathogenic vibrios causing cholera, internationally acknowledged are V. Cholerae and V. El Tor. Infections with either of them, are the ones that the International Sanitary Regulations stipulate their notification to WHO. If an outbreak of cholera-like disease occurs and the incriminating pathogen is a vibrio outside Group O-1, the health administration of the country where that outbreak happens is not under any obligation to notify WHO.

New tests have been advanced by a good number of workers to replace the haemolysis test. The ones most used are: Soda-serum agglutination and soda-sublimate precipitation of Tanamal (1959); heat and chloroform inactivation of agglutinability of V. Cholerae of Gispén (1938) and Meyer (1939); chemical flocculation test of Wahba and Takla (1962); chicken red cell agglutination of Finkelstein and Mukerjee (1963); polymyxin-B test of Gan and Tijia (1963); trypsinised broth test of Gan and Tijia (1963); and the susceptibility to Mukerjee's bacteriophage group IV (1960, 1963).

The following is a brief description of these tests:

Soda-serum-agglutination test: A high titre cholera O serum without any preservative is progressively diluted in 0.3% sodium carbonate solution. A few drops of a thick suspension of vibrio in distilled water are added to the tubes containing serum dilutions. On incubation at 37°C, cholera vibrios do not show agglutination in serum dilutions of 1:200 or above, while El Tor vibrios are agglutinated in the highest titre of the serum.

Soda-sublimate-precipitation test: It is a colloid chemical reaction in which no agglutinating serum is used. In 0.5 cc of 0.5% sodium bicarbonate solution, a few drops of thick suspension of vibrio are added and mixed thoroughly; then 0.5 cc of 0.5% mercuric chloride solution is added and the mixture shaken. Flocculation immediately forms with cholera vibrios, while El Tor suspensions remain cloudy.

Heat-inactivation of agglutinability of cholera vibrio: Suspensions of the vibrios are heated to 50°C for three hours while El Tor vibrios retain

their agglutinability, the agglutinable properties of cholera vibrios are lost.

Chloroform-inactivation of agglutinability of cholera vibrios: In this test, agglutinability of V. Cholerae by cholera O serum is lost when the vibrios were suspended in 2% chloroform. El Tor vibrios, on such treatment, remain agglutinable.

Chemical flocculation test: A 16-hour agar slant culture is washed in distilled water and the concentration adjusted turbidimetrically to 5 000 million vibrios per ml by means of a photo-electric colorimeter of the Lunatron type. This suspension is then boiled for 15 minutes. A 0.25% solution of copper sulfate is prepared and dispensed in 2-ml amounts in Wassermann tubes. Ten drops of the vibrio suspension are added, the tube is shaken once and then observed for five minutes. Any flocculation or precipitation occurring after 15 minutes is not taken into consideration. True V. Cholerae strains give a positive flocculation reaction and El Tor vibrios give a negative reaction.

Chick red cell haemagglutination test: This test, as reported by Finkelstein and Mukerjee (1963), is performed on microscope slides, across which twenty parallel lines are drawn by a ball point. A drop of physiological saline is applied with a bacteriological loop to the channels thus formed. Sufficient bacterial growth from agar cultures is emulsified in the drop to make a heavy milky suspension. A loopful of washed 2.5% chicken red blood cells in normal saline is then added and the slide tilted back and forth to mix the cells and bacterial suspension. If test is positive, clumping of the erythrocytes is detectable immediately (or at most within one minute) with the naked eye. The erythrocytes remain dispersed in negative reactions.

A modification of this test has been developed by Barua and Mukherjee, A.G. (1964). They employed organisms grown in meat-extract broth. The blood was collected in Alsever's Solution. After adding the bacterial cultures to the chick cell suspension, the mixture was incubated at 37°C for two hours and then shaken and refrigerated over-night before taking the final reading. Better detection of results was noticed by using 2% chick cells when mixed with double volumes of 24 or 48 hours old bacterial cultures.

Polymyxin-B test: Discs containing 50 units of polymyxin-B placed on the surface of the medium 20 minutes after inoculation of the medium with the test vibrio. Vibrio cholerae was consistently inhibited. Neither V. El Tor nor water vibrios were inhibited.

Phage-susceptibility test: Mukerjee, testing the bacteriophages he was able to isolate in his laboratory, found that they could be classified into four distinct groups. Testing for lysability of V. Cholerae and V. El Tor by these bacteriophages, this worker was able to prove that cholera vibrios are uniformly sensitive to group IV cholera bacteriophage while V. El Tor strains are universally insensitive to it.

Comparative studies on these tests have been carried out and it seems that there is agreement among the different workers that the following tests give the most satisfactorily consistent results: phage susceptibility, chicken erythrocyte agglutination, copper sulphate flocculation test and the polymyxin B test. The next table gives the differential diagnosis of the different vibrios in table form.

DIFFERENTIAL DIAGNOSIS OF EL TOR, TRUE CHOLERA, AND NAG VIBRIO STRAINS

Property or test	El Tor vibrios		True cholera vibrios	Non-agglutinable vibrios
	Celebes type	Zam Zam type		
Agglutination with O-1 serum	+	+	+	—* or partial
Lysis of sheep and goat cells in tubes	++	++	—	variable
Biochemical reactions of Heiberg group I	+	+	+	variable
Voges-Proskauer reaction	+++	+++	—	variable
Chicken red cell agglutination	+	+	O*	?
Tanamal soda-serum agglutination	++	++	—*	?
Wahba and Takla copper sulphate flocculation	—	—	+	?
Gan and Tijia trypsin test	++++	++++	—	—
Gan and Tijia polymyxin B test	not sensitive	not sensitive	sensitive	not sensitive
Susceptibility to phage II of Mukerjee	—	—	variable	—
Susceptibility to phage IV of Mukerjee	—	—	+	variable
Toxin production	strong	weak	variable	variable
Sayamov test in rabbit gallbladder	+	—	+	non-specific inflammation
Human pathogenicity	cholera	none or very mild	cholera	none or very mild
Resistance to environmental factors	higher*	higher*	lower	higher*

* Most often

** Frequently

*** Recently isolated cultures are +; old stock strains are often —.

After Felsenfeld, O., 1964, Bact.Rev., 28, 72.

IX EPIDEMIOLOGY

The epidemiology of cholera is an open book in certain of its aspects and is clothed with ambiguity in others.

Cholera is one of the bacterial enteric communicable diseases and as such shares with the other enteric infections the causative factors of incidence and spread. Yet cholera seems to have come to this world with an innate attribute of love to the exceptional.

Cholera stands alone among the whole group of enteric infection, endowed, so to speak, with the attribute of pandemicity.

Endemicity in most of these enteric diseases is maintained and proportioned by the "reservoir of infection" in the chronic carriers. Cholera has another story to tell. Its endemicity is intimately related to certain specific physical topographical characteristics - the low lying river deltas with shallow and slow brackish water.

Multiple clinical cases among the members of households infected is a common occurrence in diseases like shigellosis and enterica, but in cholera this phenomenon is a very rare occurrence and when it happens it is more of a manifestation of infection happening at the same time, as the secondary cases show up either on the same day or within 24-48 hours at most. In the other two diseases these secondary cases appear after a lapse of time denoting that the first case acted as the focus of infection.

Another peculiar epidemiologic phenomenon in cholera is the absolute absence of return cases arising from the return of convalescents to their homes even if discharged earlier than the agreed upon period of time for the disappearance of the vibrios from their intestinal canal. The opposite has been observed in shigellosis and enterica. The question often arises: is the convalescent carrier in cholera non-infectious? Again no medical officer of health could dare to answer the question in the affirmative in spite of the circumstantial epidemiologic evidence in favour of "yes" as an answer.

The great variation in the characteristics of the same pathogen isolated in different places, in different epidemics, and even at different times during the same epidemic - is another property not met with in the other enteric pathogens. Goodner (1963) in discussing this variability mentioned that there are at least fifty different variants among the 500 strains of cholera vibrios

in his possession. The question then arose whether the medical profession is dealing with disease caused by a single bacterial species, or that under the term "cholera" there is jumbled together a group of diseases that might be better referred to as "vibriosis".

The general principle and factors concerning the epidemiology of cholera are known and need no special exposition. They are similar to those of shigellosis, or salmonellosis in general.

However, the epidemiology of cholera comprises certain features that deserve special mention. These are:

- a. movement of population
- b. the carrier state
- c. phage typing

A. Movement of Population

As regards man's movements from infected areas to one free from infection and from free locales to infected areas, there is no doubt that such traffic of humans influences the picture of the incidence of the disease. All the history of cholera and its spread is proof enough of this as discussed before.

B. Carriers

There are two types of carriers in Cholera - the contact carrier and the convalescent carrier.

a. The contact carriers

These are persons who have been infected either from "contact" with a case, or through "contact" with the infected environment. We might term the former kind as the household carrier, and the latter category are no doubt those who have contracted a subclinical infection.

Van de Linde and Forbes (1965) report that a case of cholera was recorded in one of the employees of a big restaurant in Kowloon, Hong Kong. Sampling the 108 employees, thirty-four were found carriers of *V. Cholerae*. Twelve of these were discovered from the first rectal swab, nine from the second, seven from the third, three from the fourth, two from the fifth and one from the seventh.

The only comment to this result is that no mention is made about the intervals between the swabbing and whether these employees were isolated or left to go home. Because they were not isolated, then there is the

probability that they contracted the infection from the environment. Anyhow, the figures mentioned by these workers denote the degree of infection in the community.

The same authors report seventy carriers among 1781 contacts.

Kao (1963) reports that the carrier rate of contacts in Taiwan epidemic ranged between 0-11%. The duration of excretion of the vibrios ranged between 2-12 days.

Davies (1962) in the El Tor cholera epidemic in Hong Kong spotted 7.6% carriers among 711 isolated contacts of fifty-two cases who were mainly boat-dwellers.

Yen (1964), discussing the 1962 epidemic of cholera in Taiwan, mentions very interesting points:

- i. all carriers were hospitalized immediately on being detected.
- ii. among 1177 persons admitted to hospital on suspicion of being carriers (on what basis is not mentioned in text) 380 proved to be so.
- iii. rectal swabbing during the first month after the last clinical case revealed thirty-eight carriers among 72 000 people.
- iv. sampling further 47 000 people during the third and fourth month after the epidemic gave completely negative results.
- v. graphing the weekly occurrence of carriers by districts showed a similar pattern to that of the disease.
- vi. the ratio of active cases to carriers was roughly 1:1

It is worth mentioning that after the cessation of new cases in the 1947 epidemic in Egypt, a mass search for carriers by rectal swab was carried out. The epidemic ended on the second week of January 1948 and the survey began about two and a half to three months later. All the 94 562 samples were negative.

Chang et al (1964) carried out a carrier investigation of cholera El Tor epidemic in Chiayi in 1962. They report that the carrier rate among contacts of the ninety-seven patients was 4.81%.

These rates are compiled from the reports of the most recent outbreaks and the rates mentioned are nearly similar to those reported before 1960.

The importance of the contact carrier (both kinds) is that they are potential spreaders of infection. During the 1947 epidemic in Egypt, household contacts were isolated in hospitals or health units under observation. Out of 14 473 contacts quarantined on the same day of hospitalization of the index case, 497 proved to be carriers (3.43%). The important point about these carriers is that 123 of them were incubating the disease and showed it during isolation.

The other kind of contact carriers - i.e. persons infected through contact with the environment itself - was tackled during that epidemic by catching persons living in the infected area sneaking out of it. Out of 2 411 such migrants, forty-seven proved to be carriers and thirteen of these developed the disease.

b. Convalescent carriers

These are recorded everywhere, and up to date there is no investigation done to prove or disprove their infectiousness. The lack of reported return cases is circumstantial evidence only. The problem needs further study, and the success of producing cholera in the infant rabbit is perhaps a good opportunity to settle this question.

c. Phage-typing of V. Cholerae

Mukerjee has succeeded in developing a scheme of phage-typing of V. Cholerae. Five phage-types of this organism have been identified on the basis of their sensitivity patterns to the four groups of cholera bacteriophages.

By applying these four bacteriophages in typing of V. Cholera isolated in different countries, or different regions of the same country, it is possible to obtain information about the sources of origin and lines of spread of the disease - data of importance for epidemiological studies. For example it was found, by the application of phage-typing, that there are at least three different endemic foci in India harbouring different phage-types of V. Cholerae. In the eastern states, the phage-type 3 vibrios of the Inaba sero-type is present, while the endemic strain in South India is a phage type 1 Ogawa vibrio and the other areas have phage-types 1 and 3 and sero-type Inaba. Again, when this test was applied to the strains of the vibrios isolated in Calcutta from 1956 to 1962, Mukerjee found variations in the incidence of phage-types and sero-types of the vibrios. Up to the third quarter of 1960, phage-type 1 Ogawa

formed 80% of the isolates. These soon were replaced by Inaba type 1 and, through 1962, this latter was totally replaced by type 3 Inaba.

This variation in the phage-type of the causative vibrios in Calcutta or any other endemic area is possible and admissible because in cholera there are no chronic carriers to keep up infection with the same phage-type.

This work has been extended, and the studies show that cholera bacteriophages could be utilized with impunity for the following purposes:

- i. identification of phage-types of V. Cholerae strains for epidemiological use.
- ii. phage-typing of El Tor vibrios based on their lysis and lysogenicity patterns.
- iii. differentiation of V. Cholerae and V. El Tor strains on the basis of their sensitivity patterns to group IV cholera phage.
- iv. differentiation of NAG vibrios from V. Cholerae by their resistance to lysis by the typing phages and total insensitivity to group II phages (Mukerjee 1964).

The success and benefit from such studies prompted the World Health Organization to sponsor the establishment of a central reference laboratory for phage-typing of all the vibrios isolated in outbreaks in different areas. This, by mapping out the foci of infection of each type, would provide valuable aid in identifying the lines of spread of the disease.

Finally, it has to be mentioned that the work of Felsenfeld on the determination of circulating antitoxin and the anti-haemolysin test on populations, to investigate group immunity against cholera, has been the subject of limited studies. Thus Felsenfeld et al (1964) report that the titres of these antibodies declined after the epidemics. Aragon and Famatiga (1963) have, however, shown that the anti-haemolysin in sera of convalescents from El Tor cholera show definite titres, in contrast to the absence of the haemolysin neutralizing antibody in sera of convalescents from cholera caused by V. cholerae. These workers are of the opinion that this test could be used for retrospective diagnosis.

The above items are the ones that have been the subject of research and study in recent years in the field of epidemiology of cholera. Outside these areas, the epidemiology of the disease remains governed by the epidemiology of enteric infections in general. Cholera is one of the enteric diseases and the pathway of infection is ano-oral - a mode that indicates the extreme importance of sanitation of the environment and the practice of personal hygiene by the people.

X . ANTI-CHOLERA MEASURES

The usual title of the last sphere of discussing a communicable disease is "prevention and control". This brings up the question: how could we prevent cholera? There are two facts in answering the question - prevention at an international level and prevention at a local level. The former means eradication in a manner similar to the WHO projects of malaria eradication. This is a very long-term policy which is intimately interwoven with the overall development of the endemic foci of infection of the disease - a venturesome enterprise which public health workers visualize in their hopes for the future.

Hence, the modest title of anti-cholera measures.

The present position

Speaking from the point of view of the countries of the Region, the position is that they are being confronted, after a long era of quiescence, with one of those pandemics of cholera that wrote history up to the early twenties of this century. In fact the disease has already invaded one of the countries of the Region.

The problem is how to deal with the situation. This needs dividing the discussion into three phases.

1. Preparative period
2. The invasion period
3. Post-epidemic period.

A. The Preparative Period

All the health administrations of the countries of the Region have already been and are being informed of the news of the pandemic.

The first responsibility is to expect invasion, because it is impossible to foresee when, how and where the vibrios will smuggle themselves in. Most of the countries of the Region are endowed with vast stretches of desert uninhabited areas along its boundaries. Not only this, but most of them have residents in those areas with nomadic habits. This is a weak point in the defence to prevent invasion. In the previous pages, mention was made of spread of the disease to the different countries in the South Pacific although they were islands separated from each other by the sea.

Measures at air and seaports are commendable no doubt, but the evasive character of the disease, especially when infection is mild, makes action based

on expectancy wiser and safer. Under such circumstances territories free from infection must act on expectancy.

The following measures are recommended:

1. Members of the medical profession should be on the alert. They have to be informed of the suspicious symptomatology of the disease. Their co-operation should be invited.
2. Staff of hospitals, health centres, etc. have to be informed about the correct procedure in treatment of the cases. Accurate knowledge about the copper sulphate method of measuring the specific gravity or the dosage of bicarbonate or potassium in the infusions, etc., is pertinent.
3. The stores must be ready to supply the different units with their needs not only in saline and other compounds, but in bed accessories, in disinfectants etc. Bleaching power is indispensable, its chlorine content has to be known.
4. Anti-cholera vaccine with all the implementation that it needs, sterilizers, syringes, needles, etc.
5. Water supplies - the public ones **must** be examined to ensure their fitness. Chlorine for disinfection of the water must be available in sufficient quantities to deal with any emergency.
6. Food establishment have to be made hygienically safe.
7. Medical officers of health in the rural areas have to survey their villages to know whether there are wells or Abbyssinian pumps inside the houses - their sites as regards pollution. They must also survey the latrines of mosques and their water supplies, how the wastes of the latrines are disposed of. If possible arrangement for corrections will be started.
8. The laboratories must be ready and the staff concerned must be trained on the newer trends in the methodology of vibrio isolation and identification.

Stool specimens from cases of diarrhoea chosen at random from different parts of the country should be sent to the laboratories to examine for vibrios.

9. Organizational committees, both at the control and local levels, should be instituted. Their first job is regionalization of the treatment centres and the places of isolation of contacts, the laboratories etc.

10. Revision of the laws and regulations concerning cholera and have them ready for application once invasion happens.
11. The choice of the "post" which is going to be the seat of the "marshal" responsible for the whole activity.

The question always arises: will the public be informed of the danger if circumstances help to prevent it? There are some who answer negatively on the assumption that alerting the public of the potential danger of a disease like cholera reputed for its virulence, will invite panic.

As any anti-measures in case of invasion needs co-operation of the public, they have to be educated, taught and prepared, and this period is the best.

The motto of this period for the administration and for the people is: What is to be done and by whom.

B. The Invasion Period

This means that infection passed through.

Anti-cholera measures are the same as the measures applied in other enteric diseases with the addition of more specific ones.

These relate to compulsory hospitalization of suspected or actual cases and isolation of their household contacts; freedom of the stool of both categories from vibrios is mandatory for release. Public gatherings like fairs, moulids, etc. are to be prohibited and movement of population, especially out of the infected area, to be either prohibited or minimized and controlled. And as water is a very important vehicle of infection, all water supplies, public and private, have to be put under continuous supervision with super-chlorination of public supplies and disinfection of private ones.

C. Post-Invasion Period

This period begins after overt cases ceased to occur. The aim is to search for mild cases of diarrhoea that might be choleraic. So instructions have to be given to send to the laboratory stool specimens on rectal swabs from all cases of diarrhoea attending hospitals or dispensaries. This is to continue till the health authorities become satisfied that cholera infection has been eradicated.

Some countries do swab big numbers of individuals, searching for carriers. Hong Kong swabbed more than 3 000 and the result was one positive. In Egypt, after the 1947 epidemic, samples were collected from four villages which were heavily infected with negative results. The number of specimens was about 20 000.

Vaccination

The opinions about the effectiveness of vaccination as a control measure in cholera has been controversial for a long time. The proponents prove their case by the presence of vibrocidal and agglutinating antibodies in the blood of the vaccinated. The opponents mention that such humoral antibodies have been detected in the blood of normal people, and add that as cholera vibrios never invade the body and as the walls of the intestines remain intact, then there is no chance for the humoral antibodies to come in contact with the pathogens - hence their uselessness.

Field statistics about morbidity and mortality among the vaccinated and non-vaccinated are in favour of vaccination. To settle such an important question "WHO has launched a research programme to determine the value and improve the quality of cholera vaccine" (WHO Chr. 1965, 19, 6)

As serological investigations proved that vaccines prepared from V. Cholerae give low vibrocidal and agglutinating antibodies against V. El Tor while vaccines made of the latter organism are more vibrocidal against V. Cholerae, it has been proposed to incorporate V. El Tor in the vaccines.

Workers are investigating at present the possibility of developing an oral vaccine. Mukerjee (1963) reported finding a water strain of V. El Tor characterized by high antigenicity but with weak pathogenicity. He succeeded in immunizing the infant rabbits by the oral route, thus giving hope for developing such a vaccine for humans.

BIBLIOGRAPHY

- Barua, D. & Mukherjee, A. C. (1963) Bull. Calcutta Sch. trop. Med., 11, 85
- Barua, D. & Sack, B. (1963) ibid 11, 85
- Bhattacharji, L. M. and Bose, B (1964) Indian J. Med. Res., 52, 8
- Basaca-Sevilla, V. et al. (1964) Amer. J. trop. Med. Hyg., 13, 100
- Basu Mallik, K. C. & Roy, J. C. (1960) Indian J. Med. Sci., 14, 776
- Benenson, A. S. et al. (1964) Bull. Wld Hlth Org., 30, 827
- Benjati, C. & Keoplung, M. (1961) Amer. J. trop. Med. Hyg., 10, 914
- Beran, G. W. (1964) Amer. J. trop. Med. Hyg., 13, 698 and 708
- Bhaskaran, K., Bull. Wld Hlth Org., 30, 845
- Blackwell, Q. R. (1961) Ann. trop. Med. Parasit., 55, 321
- Burrows, W. et al. (1964) Proc. Soc. exp. Biol. (N.Y.), 57, 311
- Carpenter, C. C. J. (1963) Seminar on Cholera, Indian Medical Research Council, New Delhi, 3
- Carpenter, C. C. J. et al. (1963) Bull. Calcutta Sch. trop. Med., 11, 7
- Carpenter, C. C. J. et al. (1963a) Bull. Calcutta Sch. trop. Med., 11, 89
- Carpenter, C. C. J. et al. (1964) Bull. Calcutta Sch. trop. Med., 12, 29
- Chatterjee, R. R. & Williams, R. P. (1963) J. Bact., 85, 838
- De, S. N. (1961) Cholera, its pathology and pathogenesis, Oliver & Boyd, London
- De, S. N. et al. (1962) Trans. roy. Soc. trop. Med. Hyg., 56, 241
- Delpy, L. (1963) Bull. Wld Hlth Org., 28, 369
- Din Fon Khua (1961) Zh. Mikrobiol. (Mosk.), 32, 106
- Dutta, N. K. & Oza, N. V. (1963) Bull Wld Hlth Org., 28, 307
- Dutta, N. K. & Panse, M. V. (1962) Indian J. Med. Res., 50, 732
- Dutta, N. K. & Panse, M. V. (1963) Bull. Wld Hlth Org., 28, 357
- Dutta, N. K. et al. (1959) J. Bact., 78, 594
- Dutta, N. K. et al. (1963) Brit. Med. J., 1, 1200
- Feeley, J. C. (1962) J. Bact., 84, 866
- Feeley, J. C. & Pittman, M. (1963) Bull. Wld Hlth Org., 28, 347
- Felsenfeld, O. (1963) Bull. Wld Hlth Org., 28, 289
- Felsenfeld, O. (1964) Dtsch. Med. Wschr., (In print)
- Felsenfeld, O. (1964a) Bact. Rev., 28, 72
- Felsenfeld, O. et al. (1961) J. trop. Med. Hyg., 64, 207
- Felsenfeld, O. et al. (1964) Bull. Wld Hlth Org., 30, 833
- Finkelstein, R. A. (1962) J. Immunol., 89, 264
- Finkelstein, R. A. & Gomez, C. Z., (1963) Bull. Wld Hlth Org., 28, 327
- Finkelstein, R. A. & Mukerjee, S. (1963) Proc. Soc. exp. Biol. (N.Y.), 112, 355

- Finkelstein, R. A. et al. (1963) J. infect. Dis., 113, 99
- Finkelstein, R. A. et al. (1964) J. infect. Dis., 114, 203
- Formal, S. B. et al. (1961) Brit. J. exp. Path., 42, 504
- Freeman, B. A. et al. (1963) Proc. Soc. exp. Biol. (N.Y.), 113, 675
- Fresh, J. W. et al. (1964) Arch. Path., 77, 529
- Freter, R. (1962) J. infect. Dis., 111, 25
- Freter, R. (1962a) J. infect. Dis., 111, 37
- Freter, R. & Gangarosa, E. J. (1963) J. Immunol., 91, 724
- Freter, R. et al. (1961) J. infect. Dis., 109, 35
- Gallut, J. (1963) Bull. Wld Hlth Org., 28, 385
- Gallut, J. & Nicolle, P. (1963) Bull. Wld Hlth Org., 28, 389
- Gallut, J. et al. (1963) Bull. Wld Hlth Org., 29, 767
- Gardner, E. W. et al. (1963) J. infect. Dis., 112, 264
- Ghosh, S. N. & Mukerjee, S. (1961) Ann. Biochem., 21, 183
- Greenough, W. B. et al. (1964) Lancet, 1, 355
- Gordon, R. S., jr et al. (1964) E. Pak. Med. J., 8, 10
- Halder, D. & Chatterjee, A. N. (1962) Ann. Biochem., 22, 255
- Halder, D. et al. (1961) Ann. Biochem., 21, 213
- Hinman, E. J. et al. (1963) Bull. Calcutta Sch. trop. Med., 9, 154
- Huber, G. S. & Phillips, R. A. (1960) United States Naval Medical Research Unit Two, Res. Rept. MR 005.09-1040. 1.9, Taipei
- Hugh, R. (1962) Bact. Proc., 1, 5
- Hugh, R. (1964) Int. Bull. bact. Nomencl., 14, 87
- Kamal, M. A. (1963) Bull. Wld Hlth Org., 28, 263
- Kao, F. H. (1963) J. Jap. Ass. infect. Dis., 37, 125
- Kobayashi, T. et al. (1963) Jap. J. Bact., 18, 387
- Kovacs, N. (1963) Lancet, 2, 497
- Mackenzie, D. J. M. (1961) Report on the cholera outbreak in Hong Kong, Government Printing Office, Hong Kong
- Moor, C. E. de (1963) Trop. geogr. Med., 15, 97
- Morgan, F. H. et al. (1960) Amer. J. Hyg., 70, 250
- Monsur, K. A. (1963) Bull. Wld Hlth Org., 28, 387
- Mukerjee, S. (1963) Symposium on Cholera, Indian Medical Research Council, New Delhi, 2
- Mukerjee, S. (1964) Bull. Wld Hlth Org., 29, 763
- Mukerjee, S. (1964a) Indian J. Med. Res., 52, 331
- Mukerjee, S. & Ghosh, S. N. (1962) Ann. Biochem., 22, 73
- Mukerjee, S. et al. (1963) Ann. Biochem., 23, 201
- Naha, P. M. (1963) J. appl. Bact., 23, 3

- Noble, J. E. (1964a) J. roy. Army Med. Cps., 110
- Noble, J. E. (1964) J. Hyg., 62, 11
- Oza, N. B. & Dutta, N. K. (1963) J. Bact., 85, 497
- Panase, M. V. & Dutta, N. K. (1961) J. infect. Dis., 109, 81
- Panase, M. V. & Dutta, N. K. (1963) Arch. int. Pharm., 145, 479
- Panase, M. V. & Dutta, N. K. (1964) J. Immunol. (In print)
- Panase, M. V. et al. (1964) J. infect. Dis., 111, 26
- Phillips, R. A. (1963) Bull. Wld Hlth Org., 28, 297
- Pittman, M. & Feeley, J. C. (1963) Bull. Wld Hlth Org., 28, 379
- Pollitzer, R. (1959) Cholera, Wld Hlth Org. tech. Pub., No.43
- Sack, B. & Barua, D. (1963) Bull. Calcutta Sch. trop. Med., 11, 83
- Sayamov, R. M. (1963a) Bull. Wld Hlth Org., 28, 361
- Sayamov, R. M. (1963) Bull. Wld Hlth Org., 28, 311
- Sodhi, K. et al. (1961) Indian J. Med. Res., 49, 388
- Shrivastava, D. L. (1961) Bull. Wld Hlth Org., 25, 128
- Sprinz, H. et al. (1962) Amer. J. clin. Path., 38, 43
- Vella, E. E. (1963) Brit. Med. J., 1, 1203
- Vella, E. E. & Fielding, P. (1963) Trans. roy. Soc. trop. Med. Hyg., 57, 112
- Wallace, C. K. et al. (1964) Bull. Wld Hlth Org., 30, 795
- Watanabe, Y. & Felsenfeld, O. (1963) J. Bact., 85, 37
- WHO (1962) Wkly epidem. Rec., 37, 21
- WHO (1964) Wkly epidem. Rec., 39, 476
- WHO (1965) Wkly epidem. Rec., 40,
- Yen, C. H. (1964) Bull. Wld Hlth Org., 30, 811
- Yeoh, G. E. & Teoh, T. M. (1963) Trans. roy. Soc. trop. Med. Hyg., 57, 46