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PREFACE

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is an autonomous, international, philanthropic and non-profit centre for research, education and training as well as clinical service. The Centre is derived from the Cholera Research Laboratory (CRL). The activities of the institution are to undertake and promote study, research and dissemination of knowledge in diarrhoeal diseases and directly related subjects of nutrition and fertility with a view to develop improved methods of health care and for the prevention and control of diarrhoeal diseases and improvement of public health programmes with special relevance to developing countries. ICDDR,B issues two types of papers: scientific reports and working papers which demonstrate the type of research activity currently in progress at ICDDR,B. The views expressed in these papers are those of authors and do not necessarily represent views of International Centre for Diarrhoeal Disease Research, Bangladesh. They should not be quoted without the permission of the authors.

ABSTRACT

In this paper a review of the history and current knowledge of Vibrio cholerae and related diseases is presented. Consideration of the host defenses is made and the microbiology is discussed. Diagnostic methods and current approach to treatment are outlined. Preventive measures and approaches to epidemic cholera are also delineated.

INTRODUCTION

History:

Illness and death due to dehydrating diarrhea and vomiting can be recognised in the writings of Susruta (Sanskrit), Hippocrates, Galen and Wang-Shooho. There is still doubt about when cholera in its epidemic form was first described (1). It is likely that no long distance spread of cholera took place to Europe and the Americas before the 19th century despite clear descriptions of epidemics on the Indian subcontinent at least from the late 15th century when Portugese explorers began recounting their experiences in India. Extensions from India to neighbouring countries including China seem to have occurred. In 1817 cholera broke out with unusual severity and a high mortality in the area of the Ganges River delta. Over the next five years it spread over much of Asia and the Middle East. A second wave beginning in 1829 reached Europe and America by 1832. New York suffered heavily, and, through the predilection of cholera for the poor, made evident the extent of poverty and lack of sanitation which were closely linked to the epidemic (2). From 1817 to early in the 20th century six waves of cholera spread across the world. Since then until early in the 1960's the disease contracted in extent across the globe remaining regularly present only in the South Asia by the 1950's.

Spread of El Tor Biotype:

In 1905 Gotschlich isolated six peculiar strains of V.cholerae from the dead bodies of returned Mecca pilgrims at the quarantine camp of El Tor (3). These strains which produced hemolysins came from typical cases of cholera and agglutinated in the classical typing serum. However, not until 1961 when the "El Tor" variant produced an epidemic of major proportions in the Philippines was there general agreement that hemolytic V.cholerae could be responsible for severe epidemic human disease (4). Before 1961 the El Tor variant of V.cholerae caused epidemics only in Sulawesi (Celebes). Since then this variant has spread across Asia (5), the Middle East, Africa (6) and more recently parts of Europe. To date the American continent has been spared, although in isolated instances it has been recovered in the United States and Brazil.

There are several characteristics of the El Tor strain that make it likely that it will continue to spread across the whole world as previous strains have done. Although fully able to produce the cholera toxin and cause severe human disease the ratio of carriers to cases is much less than in cholera due to the "classical" biotype, (1:30-100 for "El Tor" versus 1:2-4 for "classical"), (7,8). In addition the duration of carriage following infection and the survival of shed organisms in the environment seems to favour the El Tor biotype. In the heartland of cholera - the Ganges Delta -

only since 1969 has the El Tor variant replaced the classical biotype (9). Thus providing for an assured continuing presence of that strain which has shown the most recent proclivity for global spread.

Enterotoxin:

The idea that the clinical manifestations of cholera were due to a toxin really has its roots in the writings of John Snow (10).

> "It would seem that the cholera poison, when reproduced in sufficient quantity, acts as an irritant on the surface of the stomach and intestines, or what is still more probable, it withdraws fluid from the blood circulating in the capillaries, by a power analogous to that by which epithelial cells of the various organs abstract the different secretions of the healthy body".

It is difficult to find a more accurate statement concerning cholera toxin and its mode of action today. This is not to say that since John Snow's time we have not made great progress. Much knowledge has been gathered confirming his brilliant insights. Enterotoxic activity in a cellfree filtrate of V.cholerae cultures was first demonstrated by De in 1953 in the ligated intestine of rabbits (11) and by Dutta and Habbu in 1955 in infant rabbits (12). Comparative studies of the ability of different strains of V.cholerae to produce toxin began with De's studies in 1959 (13). Ten years later methods for the preparation and purification of cholera toxin were established (14). Simultaneously it was shown that the toxin stimulated active secretion of chloride in the small intestine in a way that exactly simulated and competed with stimuli which increased the level of cyclic AMP in this system (15). Within a year independently three laboratories demonstrated that cholera toxin did increase levels of cAMP by increasing the activity of adenylate cyclase in intestinal mucosa of rabbits (16-18). Soon after, this was also shown to be the case in human intestine during clinical cholera (19). Since these observation there has been a rapid proliferation of studies confirming that cholera toxin activates adenylate cyclase in all tissues possessing this enzyme. This effect can be seen in intact tissue and is dependent on a specific receptor, monosialosyl ganglioside (GM_1 ganglioside) (20-21). The toxin has been characterized and contains binding subunits of 11,500 daltons (B) and active subunit of 23-24,000 daltons (A) and a bridging piece of 5 to 6000 daltons (A_2) which links A_1 to five B subunits. The current state of knowledge of the structure and function of this toxin has been well reviewed recently (22). The importance of this toxin extends well beyond the disease it produces since it has become a basic tool to assist in elucidating the way in which adenylate cyclase and the cyclic nucleotide system functions. Parallels have also been established between diptheria toxin and cholera toxin since it has now been shown that cholera toxin is able to hydrolyse NAD at the nicotinamide-ribose bond (23). Presumably there is a reaction in which a moderating protein in the adenylate cyclase system is linked to the adenine-diphospho ribose moiety.

It has also become clear that E.coli can produce a toxin which is very similar to the cholera toxin in structure and mode of action (22). Other vibrios which do not agglutinate in the classical Ogawa and Inaba Typing sera also produce an enterotoxin similar to that of V.cholerae (24). Other diarrhea causing bacteria may also produce adenylate cyclase stimulating substances as in Salmonella infections (25).

The DNA which codes for the adenylate cyclase activating toxin of E. coli (heat-labile toxin) is located in a plasmid which can be transferred to other E.coli and perhaps to other enteric bacteria (26). Close relationships between the V.cholerae genetic code for toxin, and that of other vibrios or enteric bacteria undoubtedly exist but have not been documented as yet. It has been suggested that the code for toxin in V.cholerae is chromosomally linked (27). At present it would seem wise to keep an open mind about the extent of transferability in nature of the DNA code for cholera and cholera related toxins.

THE PATHOGEN

Classification:

Vibrios are one of the commonest organisms in surface waters in the world. Their taxonomy is still undergoing rapid changes. This is particularly true with respect to those which may be associated with human diarrheal disease. The two main human pathogens are V.cholerae and V.parahemolyticus. The way in which these two organisms produce diarrhea is entirely different. V.parahemolyticus being an invasive organism affecting primarily the colon while V.cholerae is not invasive affecting the small intestine through secretion of an exotoxin.

The V.cholerae which causes epidemic human disease, however, has only three determinants which are of the O or somatic antigens (Table 1). Vibrios of other serogroups can cause sporadic human disease but have not commonly been associated with epidemic human diarrhea. Some of these can produce a cholera-like illness, however, (28,29) and do produce a cholera-like enterotoxin (24). There is considerable importance to understanding the potential relationships between the antigenic markers currently used to identify V.cholerae, the cause of epidemic cholera, and other vibrios which seem closely related which are present the world over (30).

TABLE 1

ANTIGENIC DETERMINANTS OF V. CHOLERAE

Serotype	O Antigens
Ogawa	A B
Inaba	A C
Hikojima	A B C

Relationships to Enterobacteriaceae:

V. cholerae is closely related to other members of enterobacteriaceae. The main differences are that as isolated from humans with cholera the organism is a curved rod rather than being straight. It is oxidase positive, grows luxuriantly in alkaline media in the presence of bile salts, and produces a neuraminidase which has the intriguing property of degrading gangliosides to the monosialosyl form which is the specific receptor for cholera toxin. Antibiotic resistance can be transferred between V.cholerae and other members of enterobacteriaceae.

Morphology:

The characteristic shape and single polar flogellae of V.cholerae are seen in (Fig. 1). The vibrios are short (1.5-3.0 by 0.5 micrometers). They are gramnegative and curved. The characteristic rapid motility is the basis for an immobilization test for their identification (31). In culture many variants are formed including spiral variants which had previously resulted in the classification of Vibrios as Spirillaceae.

Antigenic Structure:

Flagellar (H) antigens are shared by many vibrios and antisera prepared against them do not distinguish the vibrio causing human epidemic disease from water vibrios.

The somatic (O) antigens do distinguish V.cholerae Ogawa, Inaba, and Hikojima which are responsible for epidemics (Table 1). In most other respects the structure of V.cholerae is anlagous to other enterobacteriaceae. Antisera can be raised against vibrios which are not agglutinated by the anti

A, B or C sera (Table 1). These sera can serve to identify specific strains. A coherent internationally recognized framework for such classification has yet to be worked out but more than one hundred types are currently known. "Endotoxin" is present as in other gram negative bacteria and a number of soluble antigens including the enterotoxin are produced. There are many fewer details known of the chemical structure of V.cholerae lipopolysaccharides than is true in the case of Salmonella or E.coli but it appears to have some unique properties. Variations in the characteristic markers occur both in vivo and in vitro (32). These changes and their implications are not well understood at present, but certainly raise the question of reversion in nature of non epidemic strains to classical epidemic strains and vice versa.

Genetics:

There appear to be in addition to a morphologically distinctive group of the organisms we call vibrios, close genetic relationships as seen in DNA compositions and reassociation data (33,34) between some of them. Some organisms currently lumped together as vibrios such as V.parahemolyticus do not seem closely related to V.cholerae nor do their behaviour in human disease seem much related except that the gut is attacked. Water vibrios can also be very similar or quite disparate.

Vibrios can experience genetic changes by mutation, transformation, transduction by phage, and conjunction. There are a number of vibrio-phages which may be used for typing purposes. Knowledge of the genome and plasmids of V.cholerae is still in an embryonic state relative to other well-studied enteric bacteria such as E.coli (35).

Enterotoxin:

The enterotoxin which mediates the disease caused by V.cholerae has now been nearly completely characterized both with respect to its chemical composition, amino acid sequence, and molecular configuration (22). It is now thought to be composed of five binding "B" subunits arranged in a circular form, a linking subunit A_2 binds the active adenylate cyclase stimulating subunit A_1 to the complex. A_1 is bound to A_2 by a disulfide linkage. The molecule has been visualised (Fig. 2,3). The B subunit has been sequenced and the weight determined as 11.500 daltons and a partial sequence of the A_1 and A_2 units have been published (36). Its mode of action is quite well worked out and it bears interesting relationship to diptheria toxin in its interaction with NAD (21).

Determinants of Survival of V.cholerae:

Although other animals may be experimentally infected with V.cholerae there has been no evidence in nature of a carrier of the organism other than humans. When excreted from the body into water V.cholerae survives only for a short time (4-7 days) and in the presence of other competing bacteria even a shorter time. It does not withstand drying or mildly acidic conditions. It will survive more readily in brackish water than fresh water. Although water sources seem to be the main way the organism spreads foods have been implicated on some occasions (37). There are important differences in survival in the environment between V.cholerae "classical" and "El Tor" biotypes. The El Tor strain surviving for longer periods both in the host and in nature (4,37,38).

Strain Variations:

There are several variations that occur in nature with V.cholerae. Over a relatively short period of years serotypes shift between Ogawa and Inaba in endemic areas (1,39). In vitro similar shifts occur (39). Also a transformation to a rough variant is observed in older cultures but also in vivo (32). The crucial question that remains unanswered is whether there may be a reservoir in nature of V.cholerae that do not agglutinate in the Ogawa, Inaba or Hikojima sera which may become epidemic strains through a genetic change under the right conditions. Over a longer cycle in history there may be changes in biotype. During the last two decades we have seen the classical biotype displaced even from its well entrenched home by a variant which agglutinates in Ogawa or Inaba sera but which has a different susceptibility pattern for bacteriophages and can hemolyze red cells (39).

THE DISEASE

Clinical Manifestations:

Cholera may be present in an asymptomatic state, as mild diarrhea, or as the typical "full-blown" syndrome which will be discussed in this section. In its extreme manifestation cholera is one of the most rapidly fatal illnesses in medicine. A healthy individual may become hypotensive within an hour of the onset of symptoms and die within two to three hours if no treatment is provided. More usually the progress of the disease from the first liquid stool to shock occurs in from 4 to 12 hours with death following in 18 hours to several days. The first symptoms of cholera are an increase in peristalsis which the patient can sense as a fullness and gurgling in the abdomen. This is followed rapidly by the first loose stool

which is not of the typical "rice water" appearance that is so often referred to in cholera. After several watery movements the stools take on this typical appearance and lose any odor except a mild somewhat fishy smell.

All of the symptoms and signs of cholera derive from the depletion of water and salts from the intravascular and extracellular spaces of the body by loss into the gut lumen. The composition of cholera stool varies with its rate of loss from the body but on the average has the composition shown in Table 2. In the days before successful replacement therapy was given the

TABLE 2

COMPOSITION OF CHOLERA STOOL

When Purging Rate is 50 ml/kg/24 hrs. or More

	Concentrations (mEq or mM/L)			
	Na^+	K^+	Cl^-	HCO_3^-
Adults	135	15	100	45
Child	105	25	90	30

clinical description of cholera included detailed attention to all the stages of hypovolemic shock, dehydration and the reaction of the body in those surviving prolonged periods of poor circulation and ischemia. If properly treated only diarrhea will be seen after the acute symptoms and signs of volume loss have been relieved by replacement therapy. Intravenous replacement solutions are listed in Table 3. Vomiting is often present at the early stages of cholera and assumes particular importance with respect to the oral replacement of fluid losses. The clinical appearance of a cholera patient is shown in Fig. 4. There is little abdominal pain in cholera most of the anxiety, muscle cramps, thirst and faintness being related in their prominence to the rate of fluid loss. On rare occasions ileus may occur at the onset of illness. In such cases there may be profound shock and dehydration without diarrhea. In this situation cholera appears much as does acute intestinal obstruction. In the older literature this is known as "Cholera Sicca". It can kill particularly rapidly since the physician may lose sight of the amount of fluid that the small and large intestine can sequester.

TABLE 3

INTRAVENOUS REPLACEMENT SOLUTIONS

	Concentrations in mM/L				
	Na	K	Cl	HCO_3	Glucose
Diarrhea Treatment "DTS" solution	118	13	83	48	50
Dacca Solution 5 g NaCl "5/4/1" 4 g $NaHCO_3$* 1g KCl	134	13	99	48*	-
Ringer's Lactate "Ringers" Solution	131	13	111	29	-

* Acetate may be substituted for bicarbonate for a more stable solution.

Initially there is no real difference between cholera and the acute watery diarrheas that are due to enterotoxigenic E.coli or 'non cholera' vibrios. If cholera is not treated by an antibiotic, however, the diarrhea will continue for a longer time with sustained large volumes lost. A picture of a patient surrounded by the bottles required to treat him is seen in Fig. 5. At the most severe extreme of the spectrum of disease it is not unusual for an individual to purge 100% of his or her body weight in 4 to 7 days of diarrhea (40).

Complications which occur in cholera can be predicted from the nature of the disease with one exception which, in the case of children, is most important. Altered consciousness is the rule in cholera but the mental state is one of being in a somewhat detached state. Cholera patients even without a detectable blood pressure can be aroused and give lucid and accurate information as to time place and person. In children one not infrequently may see unconsciousness or convulsions. This is frequently a warning of hypoglycemia. Since this has obvious therapeutic implications

one must be alert to it. Hypoglycemia can also occur with little change in consciousness. The cause of low blood glucose is far from clear and is not related necessarily to states of severe malnutrition (41).

Electrolyte imbalances are the next most common complication and also may present as alterations in consciousness that cannot be accounted for by the severity of the diarrhea. Most common in children in the tropics is hypokalemia. Its manifestations include intestinal ileus, weakness and cardiac arrhythmias. If inappropriate solutions have been given at home or elsewhere there may be hypernatremia or water intoxication. Acidosis if not attended to in severe cases can result in a fatal syndrome that is difficult to treat (42). Renal failure is most often associated with inadequate hydration and the prior administration of a pyrogenic solution or various drugs and stimulants before rehydration. It is usually reversible without dialysis.

The combination of depressed states of consciousness and vomiting leads to a high risk of aspiration with its sequellae. Care must be taken while treating such patients to avoid this risk.

Pathophysiology:

Cholera is a topical disease, the organism, V.cholerae, never actually entering any tissue of the body. It is swallowed either with water or food. It must pass through the stomach in order to colonize the small intestine even though it is extremely acid sensitive. In the small intestine it must find adequate culture conditions to proliferate. There are several characteristics of pathogenic V.cholerae that are important determinants of the colonization process. These include motility, chemotaxis, toxin production and an as yet unknown colonization adhesion factor (43,44). Once the organism has penetrated the mucous layer and begun to colonize the lining epithelium of the gut the intestine begins to secrete an alkaline, bile-rich solution which provides ideal growth medium for the vibrio. The toxin secreted by the vibrios binds tightly to the lining cells and continues to exert its effect for many hours. It is not washed away once attached to its tissue receptor even when there is specific antibody present in the solution.

The mechanism of action of the toxin has been discussed but not its cell specificity in terms of its secretory effect. It is likely that the main source the secretion in the gut are the crypt cells of the small intestine. The villus tip cells are mainly occupied with absorption. The actual mechanism of fluid loss is still not entirely clear and demands a full knowledge of the normal mechanisms of ion traffic in the different cells lining the gut. Probably all levels of the intestine are affected by the cholera toxin. What is seen as cholera stool is the net end result of non-specific stimulation of adenylate cyclase in all epithelial cells. The duodenum has the least absorptive capacity, so per unit length the most

fluid is lost from this segment (45,46). The colon has the greatest absorptive capacity and least secretory capacity and contributes the least to the volume lost in cholera.

In addition to fluid losses it is very likely that the cholera toxin triggers a variety of other phenomena. Since the intestine is a very complex endocrine organ and many hormones are governed by the levels of cyclic nucleotides in the tissues that secrete them, it is likely that cholera toxin disorganizes the delicately integrated humoral systems that coordinate normal intestinal function in ways that have not as yet been defined. It has been noticed both grossly by the mucous flecks in the stool which account for the "rice" of the "Rice-water", that the discharge of mucous from the intestinal goblet cells is due to cholera toxin. There is some data that indicates that there is a decrease in the ability to produce gastric acid in cholera patients (47). This could either be that the vibrio tends to select individuals with low ability to produce acid or that the disease affects the regulation of gastric acid secretion. Thus although the main features of fluid loss in cholera have been worked out to a considerable degree there are still many features yet to be described. These are contingent upon an improved knowledge of the humoral control mechanisms of the intestine.

Treatment:

The treatment of cholera is extraordinarily simple both in concept and execution. The water and salts lost in the cholera stool must be replaced in comparable amounts and concentrations. The replacement can, except in the most severe cases, be accomplished by mouth. The solutions used are listed in Table 4. It cannot be emphasized strongly enough that the earlier replacement can be begun the less chance there will be for complications secondary to severe volume depletion to occur. Basically for watery diarrhea everyone should have available to them packets of salts of the correct amounts to mix with a given volume of water to initiate treatment as soon as it is apparent that the diarrhea is of a sufficient severity to threaten. This treatment does not require a physician, nurse or health worker of any sort. It demands that ordinary individuals who may be at risk have sufficient knowledge to do the appropriate thing. If the packet of the appropriate mixture of salts is not available then table salt and sugar may be used according to the amounts listed in Table 4, until the proper mixture is obtained. All except the most severe cases can be managed by oral replacement therapy alone.

Intravenous therapy is needed by an increasing number of patients when the volume of stool output exceeds 100 ml/kg/24 hours or 7 liters a day in a 70 kg. person. The other place intravenous therapy is needed is when appropriate oral rehydration has not been given and a patient has been allowed to slip into shock (48). The solutions that may be used are listed in Table 4. When a patient is in shock and has been heavily purging a true

TABLE 4

ORAL REPLACEMENT SOLUTIONS

		To Add : Grams per liter Drinking water	Concentration mM/L				
			Na^+	K^+	Cl^-	HCO_3^-	Glucose
WHO SOLUTION	NaCl	3.5	90	20	80	30	111
	$NaHCO_3$	2.5					
	KCl	1.5					
	Glucose	20					
HOUSEHOLD SOLUTIONS	NaCl	5	85	-	85	-	111
	*Glucose	20					

* May substitute sucrose 40 grams/L.

emergency exists. An estimate must be made of the amount of fluid lost. Table 5 outlines the rough guidelines for doing this. Initially the deficit must be given as rapidly as is possible through a number 18 needle in a large vein. In this way about 1000 ml. can be given in 10 to 15 minutes and full initial hydration can be accomplished within an hour in most cases. The initial period is the most critical and time should not be wasted in trying to do cut downs. Any vein that can be entered quickly may be used. In our experience in Dacca the order of preference is as follows: first, peripheral arm veins - if not too constricted; second, external jugular veins (both in adults and children); third, femoral vein; and fourth, in infants, scalp veins. The femoral vein can serve for very rapid infusion of the first one or two liters until a more stable location is available. Care should be taken to insure a good preparation of the skin since infection can result. Even when a patient appears to have no heart beat it is worth carrying out a rapid infusion because in near terminal cholera patients the heart is inaudible and the pulses virtually absent. During therapy the peripheral and central pulses should be monitored to guide the initial rates of

TABLE 5

CLINICAL FINDINGS TO ESTIMATE FLUID VOLUME DEPLETION IN CHOLERA

Finding	Depletion percent body weight		
	0-3	4 - 8	8 - 12
Central pulses (femoral, Carotid)	Full	Full	Weak
Peripheral pulses (radial, pedal)	Full	Weak	Absent
Skin Turgor	Normal	Decreased	Poor
Eyes	Normal	Slightly sunken	Sunken
Muscles	Normal	Some cramps	Severe cramps
Appearance	Alert Slight thirst	Alert thirsty	Restless very thirsty
Urine flow	Normal	Reduced	Absent

infusion. The blood pressure is of little use because peripheral arteries are constricted while strong central pulses remain. The lung bases should be observed for signs of congestion particularly in patients which have been neglected and have had long sustained acidosis (42). When initial hydration has been successfully done then the fluid losses must be matched. In severe cases it is best to leave the intravenous line in place while it is being determined whether the patient is able to keep up with his losses by drinking the oral replacement solution.

The most important life threatening complications of cholera to recognise early is the presence of hypoglycemia. Any alteration in the state of consciousness particularly in a small child or baby must be assumed to be due to this and treated according to the best methods for managing hypoglycemia. This involves the intravenous infusion of 3-4 ml/kg of 25% glucose as a bolus then initiating glucose in the infusion to provide 10 mg/kg/hour. Blood glucose should be tested and followed if possible in such instances.

Electrolyte imbalances are the next most common problems in the treatment of cholera. They usually present clinically as alterations in the state of consciousness or convulsions especially in the case of a low or high plasma sodium. In acidosis there may also be, in severe cases, alterations in consciousness, severe hyperventilation, often with symptomatic respiratory distress, and restlessness. In the case of potassium depletion there may be irritability or even some obtundation, ileus that results in marked abdominal distention and an inability to take replacement solutions by mouth, and a marked weakness. For accurate diagnosis and treatment of these complications it is very useful to follow the serum electrolytes. Each should be corrected accurately but at a gradual pace to avoid rapid shifts in salts and water across tissues such as the brain to avoid the catastrophic complications associated with cerebral edema.

Renal failure should be treated conservatively. In most instances there may have to even be early modest potassium replacement in the face of anuria to protect against cardiac arrhythmias. Dialysis is rarely necessary and patients are usually able to eat sufficient calories as carbohydrates to minimize the breakdown of tissue proteins.

Ordinarily cholera has been seen in recent years in parts of the world where atherosclerosis is rare. When this disease has struck populations in Europe such as in Italy or Portugal there is an added risk of infarction of heart, brain or kidney due to poor perfusion across diseased arteries. In such situations early and effective replacement therapy has even a greater urgency than otherwise. This is of particular note since when cholera enters a new nonendemic area the attack rate is similar across all age groups. In an endemic area mostly young children are affected.

Methods of Diagnosis:

It is not necessary to make a bacteriologic diagnosis to treat cholera or the related watery diarrheas because the fluids lost from the intestinal

tract have approximately the same composition given an equivalent rate of stool output regardless of the etiology. Clinical diagnosis rests on the history of acute onset and the watery stool in the absence of high fever or much abdominal pain. Treatment is based solely on estimating the degree of dehydration.

The stool when examined microscopically will not show any very distinctive features. Limited numbers of white cells and red cells may be seen and where there is endemic parasitic disease one may find the prevalent ovae and helminths or protozoae. The most effective rapid means to recognise cholera specifically is by means of a dark-field or phase microscope. If cholera stool is viewed under darkfield conditions the vibrios are seen in large numbers with a characteristic motility that gives the appearance of shooting stars. If no antisera are used and one sees the characteristic vibrio it is not possible to distinguish those vibrios that agglutinate in the Ogawa or Inaba antisera from a vibrio which does not. If there is an epidemic it is most likely that true V.cholerae is what is seen since the so called non-cholera vibrios do not cause epidemic disease. If the classical antisera for Ogawa and Inaba are available the rapidly motile vibrio will be totally immobilized by the addition of the specific homologous antiserum and an immediate specific diagnosis can be made (31).

Cultures can be made either directly from stool or from a rectal swab. There are a number of media on which V.cholerae will grow readily. These are listed in Table 6. There are advantages to each of them. However, it seems that the best compromise is for a relatively inhibitory medium that will discourage the overgrowth of other microflora and a medium from which colonies can be picked and directly tested in a slide agglutination test. In addition to V.cholerae wher one is trying to find out what may be the cause of disease in a clinical case which occurs not during an epidemic it is also desirable to look for non-cholera vibrios (28), and enterotoxin producing E.coli. Unfortunately neither of these organisms can be readily recognised as a pathogen without resorting to elaborate and controversial serologic reactions or to testing for the ability to produce enterotoxins.

HOST DEFENSE

Nonimmune Defenses:

In order for V.cholerae to deliver its toxin to the lining cells of the small intestine it must traverse several potentially formidable barriers. The first of these is gastric acid. It has been shown in volunteers who have been fed vibrios that there is a difference of one million fold in the dose required to establish disease in an individual with gastric acid as compared to one who has had that acid neutralized (49). It is likely that were acid

TABLE 6

Agar Medium	CHARACTERISTICS		
	Inhibitory	Colony appearance	Direct slide agglut.
Gelatine	No	clear hazy ring	Yes
Meat extract	No	clear-greyish	Yes
Mac-Conkeys	Yes	Clear	No
Monsur	Yes	black centres	Yes
TCBS	Yes	Yellow	No

secretion stimulated as by histanine, the stomach would become virtually an absolute barrier to cholera. The importance of acid is further underscored since in situations where sporadic cases are occurring persons who have undergone gastrectomy or who are achlorhydric have higher attack rates (50).

The next barriers are a complex of propulsive gut motility, the mucous lining layer, and a host of enzymes and bile salts. V.cholerae has particular adaptations that permit it to thrive under these conditions which tend to inhibit the growth of almost all other bacteria. The vibrios' combination of very active motility, a mucinase, chemotaxis directed toward the gut mucosa, and proteases all combine to allow this organism to colonize very successfully in the small intestine.

Those organisms that do not become established in the upper intestine then must compete with other microflora of the lower gut. The conditions in the terminal ileum and colon are unfavourable to vibrio growth. The acidic end products of anaerobic metabolism in the caecum rapidly destroy

V.cholerae. Thus it is possible to have an infection of this organism in the jejunum and not detect it in the stool. This fact should be remembered when interpreting information on carriers based upon information from stool cultures.

Systemic Immune Defenses:

After natural infection by V.cholerae circulating antibodies can be detected against several antigens including the toxin. Bacterial agglutinins were first detected in the sera of convalescent cholera patients (1). Now antibodies to the specific somatic, or O antigens, can be demonstrated in a variety of ways which include direct agglutination of heated V.cholerae, the agglutination of chicken red blood cells which have been coated with antigens a vibriocidal test with the end point of vibriolysis which is complement dependent, and other complement fixation tests. In addition to the more specific O antigen - antibody reactions, antibodies to the H or flagellar antigens can also be detected after cholera. This antigen being shared with many other enteric organisms is of no diagnostic value. All of these antibodies can also be raised by parenteral injection of antigens as vaccine components (39).

Vibriocidal antibodies reach a peak at 8 to 10 days after onset of clinical illness, then decrease returning to baseline at from two to seven months later (51). The vibriocidal response correlates with resistance to infection (52) but as may be seen later may not be the main mediator of protection. The O antigens are determinants of vibriocidal titres and particularly in the case of vaccines may have some type specific properties.

With the discovery and purification of cholera toxin the measurement of antitoxic immunity became possible. After natural infection patients develop antibodies to toxin. Protection does accrue from circulating antitoxin but probably not at the level induced by natural disease. Protection by circulating immunity to toxin has been shown both by parenteral immunization of animals by toxin or toxoid (53) and by passive transfer of a hyperimmune serum pool to nonimmunized animals. In a natural setting for cholera, however, there is no correlation between antitoxic levels and incidence of the disease (54).

The early response to somatic antigen after natural infection is in the IgM class. Subsequent challenges by either natural or parenteral vaccine antigens tend to produce an IgG response. Both responses decay after peaking at 7-14 days after challenge. The role of circulating antibodies in protection against natural disease is not clear despite the correlation of vibriocidal antibodies with incidence of disease in an endemic area (52). From experimental work it would seem likely that only IgG would be filtered to any extent across epithelium to the intestinal lumen, and it is only effective if titres are very high (53). However, both IgG and IgM have been demonstrated in the gut lumen and shown to possess activity against cholera antigens (55). It is likely that the only significance of circulating

antibodies except when present in high titres is as epidemiologic markers of an infection or parenteral immunization.

Local Immune Defenses:

Since cholera is entirely a topical infection it would seem most likely that topical defenses would be the main determinant of protection against infection by V. cholerae. It has been known that recurrent infections are rare in cholera (56). Also in endemic areas the incidence of disease decreases rapidly with age (57). Thus there seems no doubt that highly effective immunity does occur in cholera. This has been further documented by volunteers studies in which individuals who had previously been challenged with V.cholerae and had contracted the classical illness were rechallenged after a period of 3-6 months. Such people proved highly resistant to re-challenge despite rather low circulating antibody titres (58). Thus it seems clear that there are powerful local immune defenses in the gut that are marshalled against cholera. Early work recognising local antibody in contrast to serum antibody as a potentially important protective defense against experimental cholera carried out by Burrows has been reviewed (1). From this awareness has evolved a more detailed knowledge of how local immune mechanisms in the intestines function. Antigens are detected by the Peyer Patch lymphocytes. These immature cells then migrate through the lymphatics to the circulation and are "processed" at a site that has not been fully clarified as yet. The "processed" lymphocytes then return to the gut, and perhaps other tissues, and tend to locate themselves in areas where antigen is present. Their product once back in the gut mucosa is secretory IgA. Studies using cholera toxin as a "probe" have allowed a description of this immunocyte traffic as it pertains to cholera (59).

The question of how local secretion of IgA or the presence by exudation from the serum of IgG or IgM can mediate destruction of vibrios in an environment where complement does not function has challenged the ingenuity of investigators. There is no such problem with respect to how antitoxic antibodies may work. Clearly if there is a layer of antibodies adjacent to the epithelium that will bind and deactivate toxin before it attaches to the cells thus will prevent all of the disease manifestations. Thus in the surface of the intestine such antibodies may play a very important role in defense against clinical illness if not against infection itself. It is possible that failure by the upper intestine to secrete in response to toxin limits the growth medium available for the multiplication of V.cholerae so in fact antitoxic antibodies may at least limit the numbers of vibrios in an important way. Several mechanisms by which the growth of V.cholerae can be inhibited are likely. The process by which vibrios attach to gut epithelium is highly specific (60) and antibodies against whole vibrios interfere with this process. Motility is important in pathogenesis and antibodies against whole vibrios or specific O antigen will cause clumping and arrested motion. It is unlikely that phagocytosis or vibriolysis are important in the gut

lumen. The importance of antibodies to other specific vibrio traits associated with pathogenicity such as mucinase are not yet known.

Measuring antibodies at the mucosal surface of the intestine is very difficult. The mucous layer overlying the epithelium may sequester antibodies within itself and hold them close to the gut surface. The contents of the gut lumen are heterogeneous and highly proteolytic. The problem is to measure the antibodies that are present within several microns of the cells of villus tips and crypts. At present satisfactory methods have not been developed to do this. Most of our current knowledge has been derived from observing the traffic of immunocytes (59) and from challenge experiments (58).

Vaccines:

Since natural infection confers quite effective and long lasting immunity against cholera, it seems reasonable that a vaccine could be made to elicit this protective immunity without disease. Early efforts were directed at preparing vaccines from killed whole vibrios which were injected parenterally. Critical tests of these vaccines did not occur until the early 1960's and showed that some protection could be achieved in populations where cholera was endemic. At best 90% protection was seen in the most potent vaccines used. This immunity waned rapidly such that only a trivial residual effect was present after a year (61). Recently aluminium adjuvanted vaccines have extended the duration and quality of protection of whole cell vaccines (62). Purified polysaccharide fractions of the antigens specific for serotypes have also been used. Inaba polysaccharide and whole cell mono specific Inaba vaccines protected against infection by V.cholerae, Inaba El Tor biotype. A monospecific whole cell Ogawa vaccine did not give protection in the same epidemic (63). In another trial in the Philippines significant protection was conferred by a monovalent Inaba whole cell vaccine against Ogawa infections (64). Thus it would seem that Ogawa vaccines do not protect against Inaba infection but Inaba vaccines do cross protect against Ogawa infections. This was in spite of a considerable vibriocidal titre against Inaba that was generated by the Ogawa vaccine, again suggesting that the vibriocidal titre itself is only an accidental correlate of protective immunity.

The addition of adjuvants was initially fraught with difficulties because of a high incidence of local reactions observed when an oil-adjuvant whole cell vaccine was used in the Philippines. More recently reports of aluminium phosphate adjuvanted vaccines which were tested both in the Philippines and Calcutta seem promising with appreciable protection sustained for up to two years (58).

The discovery and isolation of cholera toxin made possible the preparation of toxoid vaccines. To date fairly extensive animal testing has

been done with formalinized and gluteraldehyde deactivated material, and a field trial has been carried out with the gluteraldehyde toxoid (65). This demonstrated a very low level of protection but seems to have been a poor antigen. A new candidate toxoid vaccine has been prepared recently by Swedish workers without the need of deactivation. This is based on isolated and purified binding (B) subunits. It is likely that any toxoid would be combined with lipopolysaccharide or whole cells and adjuvants for the theoretical maximum effects. To date no such vaccine has been prepared or tested and this avenue presents the most feasible immediate route of vaccine development.

With the knowledge that the most solid protection against cholera both in volunteers and in nature is conferred by a previous infection and that this protection is evident despite a low level of circulating antibodies. There is a major effort underway to develop a live vaccine strain of V. cholerae. The ideal specifications for such a strain would be an organism that possessed all of the pathogenicity factors that allowed it to colonize the small intestine (motility, chemotaxis for gut epithelium, mucinase, adhesion factor, etc.) but that did not produce a complete toxin molecule. It would be desirable that it produced the binding ("B" or "light" chain) of the toxin since this does not produce diarrhoea or damage and would elicit antibodies against itself, thus developing a solid local prevention for binding of the complete toxin molecule. The most rational and effective way to go about preparing such a vaccine strain would be by modern genetic engineering methodology. As yet this has not been permitted. In lieu of this, strains must be sought in nature or by the action of mutagenic agents. Originally in 1963, Mukerjee isolated four strains of water vibrios from the Middle East and seven strains from Calcutta. The most promising of these strains has been given to humans without causing disease but is known to produce a low level of toxin. It does elicit antitoxic antibodies in infected hosts. Because of this it seems likely that reversion to full toxigenicity might occur in a large scale trial so this has not been done. The same has been true of a mutant generated by mutagenic agents in the laboratory by Finkelstein more recently. Bhaskaran and Sinha first applied mutagenic methods to generate vaccine strains, however, the strain they chose to test probably lacks a colonizing ability and for this reason has not been pursued (66). Most recently another strain generated by a mutagen has been reported and characterized as a vibrio which produces only B fragments of the toxin (58).

At present it is likely that in the near term we shall see aluminium adjuvanted whole cell, lipopolysaccharide and toxoid vaccines in various mixtures and combinations. In the more distant future when there is more confidence in the control over techniques of genetic engineering an effective and stable oral vaccine strain is likely. In the meantime it is possible that there would be very lucky accidental mutant from mass screening of cultures exposed to various mutagens that would be stable, not produce active toxin, colonize readily and induce solid protective immunity.

EPIDEMIOLOGY AND PREVENTION

Manner of Spread:

It should be stated at the outset that here are still many unanswered questions about the spread of cholera. The epidemic disease even in endemic areas is characterized by periods between outbreaks of many months during which there is no evidence of V.cholerae in water or food, there are no animals or vectors known, and by rectal swab methods no human carriers in the midst of large populations surveyed. This is not to say that long term human carriers do not exist -- they do, but they are rare. Where then does V.cholerae make its home between outbreaks? One possibility is that the large reservoir of V.cholerae NAG present in surface waters throughout the world can, given certain circumstances, undergo genetic change to become a fully virulent epidemic strain. This could occur by a variety of routes which include plasmid exchanges, transduction by phage, transformation or mutations. Since mutation is discrete and rare it is the least likely mechanism. Since ability to detect human carriers is not very good and knowledge of vibrio genetics not very advanced it is not possible to discriminate between these possibilities.

During epidemics there are many cases purging large volumes of vibrio-rich stool which usually finds its way into the water that is used for washing, swimming, cooking or drinking. The definitive observations on the spread of cholera by water were made by John Snow in England during the epidemic of 1832 (10). In most situations to the present water seems the principal route to epidemic spread. Food has also been implemented in some epidemics but it is often through the use of contaminated water at some point in the preparation process (57). Although the El Tor biotype has a longer survival in nature outside of the host than classical strains it is still fragile and does not last as long as other enteropathogens. It can be said that except in the case of very special conditions where the water is alkaline (ph 7.5-8.5), temperature is cool, shady and free of competing bacteria the life span of V.cholerae outside of the human host is usually less than 5 days. In food survival is even more limited, however, some shellfish if refrigerated can provide a vehicle that is worrisome. These have played a role in out breaks in the Philippines, Thailand and more recently Italy (67).

As has been pointed out vibrios that appear identical to V.cholerae, Inaba or Ogawa but lack the O specific natigens continuously inhabit surface waters the world over. At present with the exception of the possibility of human carriers these seem the most plausible reservoir from which epidemic strains could spring.

Human carriers do exist and in some instances can shed vibrios for a very long time even years. Up until 1959 the idea that such carriers played

an important role in the spread of disease was not given much credulity (68). All seem to agree that people with mild illness or in convalescence are important during the epidemic spread of cholera. As has been discussed, however, during a full outbreak there is generally little mystery about how the disease is spreading since there are many cases and a variety of opportunities by which water and food are contaminated. With the advent of the El Tor biotype more importance has been given to human carriers, since this variant persists in man longer and produces more subclinical infection than did classical strains.

There are several important problems about the detection of human carriers. If vibrios are sequestered in the gallbladder or upper intestine they may not be found in stool since acid conditions in the colon will kill them. Thus a carrier may be silent, only detected if intubation of the upper intestine or if a purgative is given that is strong enough to result in an alkaline stool. For this reason the rarity of long term carriers may be over estimated. Another approach has been to screen the sewerage effluents from populations for V.cholerae. During interepidemic periods in Calcutta positive isolates have been found (69). There seems no doubt that a role is played by transient human carriers or mild cases during epidemic periods. It is possible that the few long term carriers may explain sequestration in interepidemic periods as well. More attention is needed both to this question as well as the potential for a pool in nature of vibrios that are under appropriate conditions converted from innocuous water vibrios to pathogens capable of epidemic spread.

The pattern of outbreaks has indicated that the greatest risk of disease is among household contracts of symptomatic cholera patients (70). There is a qualitative difference in attack rates between populations that have continuing experience with cholera and those which do not. In cholera endemic zones the extent of infection is great (71), and the highest incidence is in children sparing the first two years of life. In areas not experiencing cholera all age groups are equally attacked. There may be differences in attack rates dependent on which individuals in the households are most in contact with soiled clothing, cleaning of stool etc. These may vary according to the cultural settings of the disease. Common source outbreaks do occur and depend upon such things as contaminated bottled water (Portugal 1974) or Shellfish (Italy 1972).

Prevention:

It is clear that if there were no opportunities for water or food to be contaminated with cholera vibrios, there would be no spread of the disease. This means that with adequate attention to sanitation and hygiene cholera is not a problem. Much of the world as yet does not have a basic human waste disposal system that functions. Even in wealthy countries with excellent technology examples of raw sewage mingling with surface waters are not rare.

Thus few areas of the world are truly inaccessible to the spread of cholera. With the gradual march of the El Tor biotype out of Asia across the Middle East, to Africa and Europe over the past two decades it should be apparent that all parts of the world will be affected by the present pandemic.

Surveillance of cases of diarrhea or of sewage are perhaps the best early warning methods. If one awaits a cluster of cases of full severity then there will be a rather large community reservoir. In the case of El Tor variants as many as 100 asymptomatic or mild cases exist for each severe illness (57). When a case is located if it is an isolated instance, as in importations from affected areas, quarantine procedures may be of help. Most important is to be sure that the disposal of stool is into a system that effectively decontaminates this waste or that vibriocidal agents are used prior to disposal. In most situations early warning is lacking and the disease is already widespread in the community when discovered. Furthermore the communities attacked are poor, crowded and characteristically without adequate disposal of human wastes. In such situations few measures have proven effective although many are used and have had claims made for them. It can be said that since the epidemic curve of cholera outbreaks is characteristically abrupt, that by the time public health measures are actually applied the epidemic has peaked and is waning. Thus any measure will appear to be highly effective and everyone is satisfied.

Let us look at a few of the measures advocated. Boiling of water for all purposes is clearly an expensive undertaking since it requires a considerable quantity of fuel. When such a program is recommended the authorities should be ready to provide the needed energy source for doing it, otherwise it is usually out of reach economically for the population most at risk -- the poor. Clean water sources such as by means of deep tubewells may be provided but unless these can produce sufficient water to supply all needs there will be little impact. It does no good to provide a safe water source for drinking and cooking if people bathe and wash household articles in contaminated water. Perhaps the simplest and most overlooked measure during an epidemic is effective hand washing which at least may cut down cross contamination within a household where cholera cases are present. Care should be taken when a community is known to harbour cholera that any food products or water that will be used by other communities is not contaminated. Flies may carry V. cholerae from contaminated areas but are not a vector of proven importance.

Immunization campaigns with present vaccines are costly and relatively ineffective. They do not reduce the spread of disease only the incidence of clinical cases.

In rural areas of developing countries most of the methods commonly advocated cannot be implemented. Treatment however, is very simple and highly effective. The major emphasis should be placed on this. It should

not be neglected in favour of control measures which are of doubtful effectiveness due to inability to truly implement them.

Role of Treatment:

At the present time since vaccines are not very effective and control measures during outbreaks are also of unproven value the central importance of inexpensive simple and effective treatment seems obvious. With proper management there is no reason for any patient to die from cholera. Although the vast majority of patients will do very well on oral rehydration therapy alone, particularly if started early, there will always be some patients who will require intravenous fluids with the expertise and equipment necessary to give it effectively.

In the affected communities there should be a major effort to teach people how to mix and give oral rehydrating solution. If no packets of the ideal composition are available, instructions should be provided on how to mix table salt and sugar with an appropriate volume of water. As soon as a mixture which includes bicarbonate and potassium can be provided this can be readily substituted because the method of measuring how much water to mix with salts and sugar are the same for the simple mixture of home ingredients or a packet provided from outside.

A treatment centre should be established where seriously depleted cases can promptly receive intravenous fluids. This requires 24 hours coverage by trained staff and proper solutions, tubing and needles. In severe epidemic situations patients can be managed very effectively simply by observing the pulses and appearance without further measurements. Preferably cholera cots with buckets and simple but accurate observations on intake and output will be possible (Fig. 6).

Tetracycline or another effective antibiotic should be used in all patients suspected of cholera as it will shorten the diarrhea and reduce the need for intravenous and oral fluids. In addition use of an antibiotic will decrease the potential for contaminating the environment.

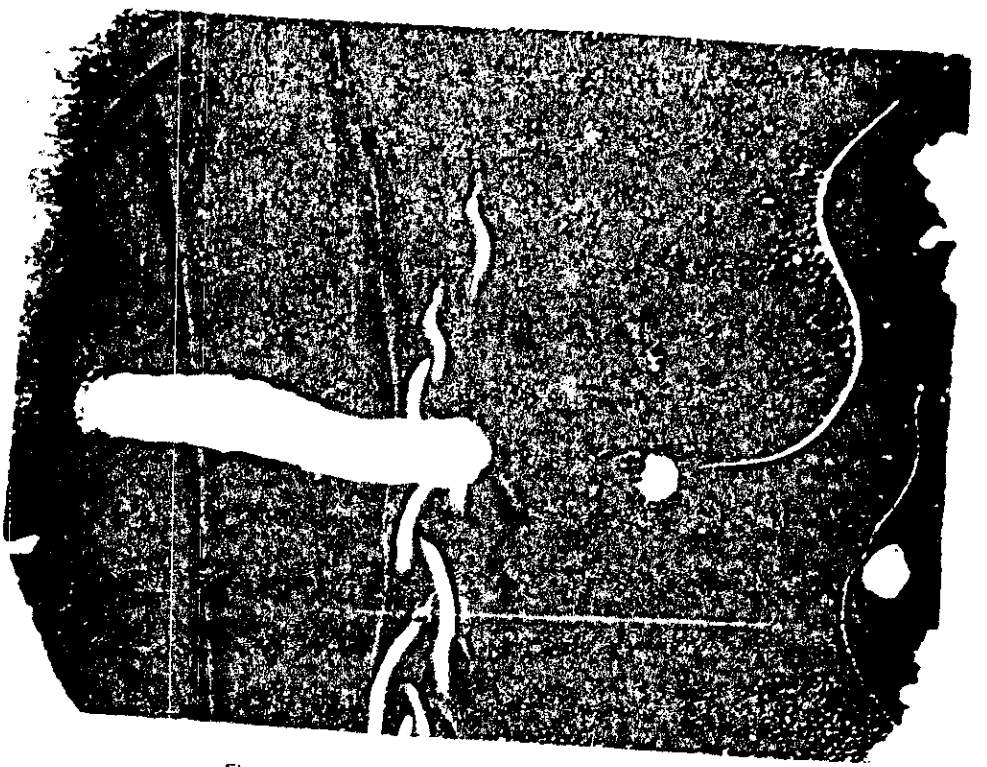
Effective sterilization of all stool and vomit is essential before disposal to avoid any chance that the treatment centre be a source of spread of the disease. Despite such measures in rural areas where patients are brought in small boats, the area around a treatment centre will be contaminated and people in the neighbourhood should be protected in all ways possible. It is likely that taking the patient out of his community to a treatment area will reduce the contamination of the home community while somewhat increasing the risk to the area near the hospital.

Anticipation and planning how to manage epidemic cholera is very important since the volume of fluids required and the fulminant nature of the disease usually serves to frighten and disorganise even modern facili-

ties. Improvisation is usually needed. Because of the speed with which cholera may kill and the simplicity and effectiveness of treatment, locating centres near the areas affected is particularly important even if sophisticated facilities are available but several hours of travel away.

FIGURE I

VIBRIO CHOLERAE



Electron micrograph : × 50 000.

FIGURE 2

ELECTRON MICROSCOPY OF CHOLERA TOXIN

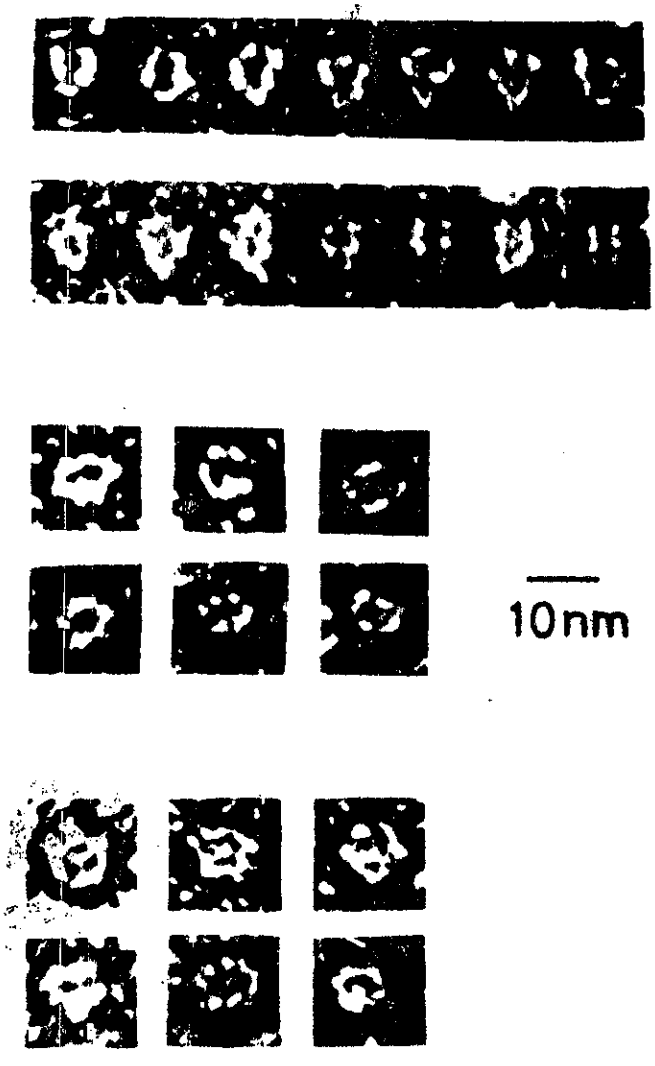
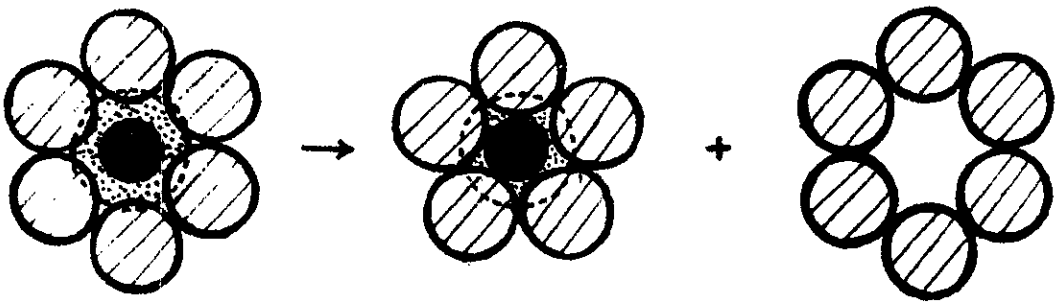


FIGURE 3

POSTULATED MODEL FOR CHOLERA TOXIN



Choleragen
AB_6

Choleragen
AB_5

Choleragenoid
B_6

FIGURE 4

CHOLERA PATIENT BEING ADMITTED BY DR. A.K.M. JAMIUL ALAM TO CRL 1964

SUNKEN EYES

POOR SKIN TURGOR

FIGURE 5

RECOVERED CHOLERA PATIENTS WITH BOTTLES OF INTRAVENOUS FLUID REQUIRED TO MAINTAIN HIM DURING HIS ILLNESS

LEGEND FIGURE 6

Several simple cholera cots for patient comfort and case of measuring stool output during cholera.

A. Camp cot with hole on which plastic sheet and sleeve channels stool to calibrated bucket.

B. Camp cot with hole on which plastic sheet can be placed to channel stool to bucket.

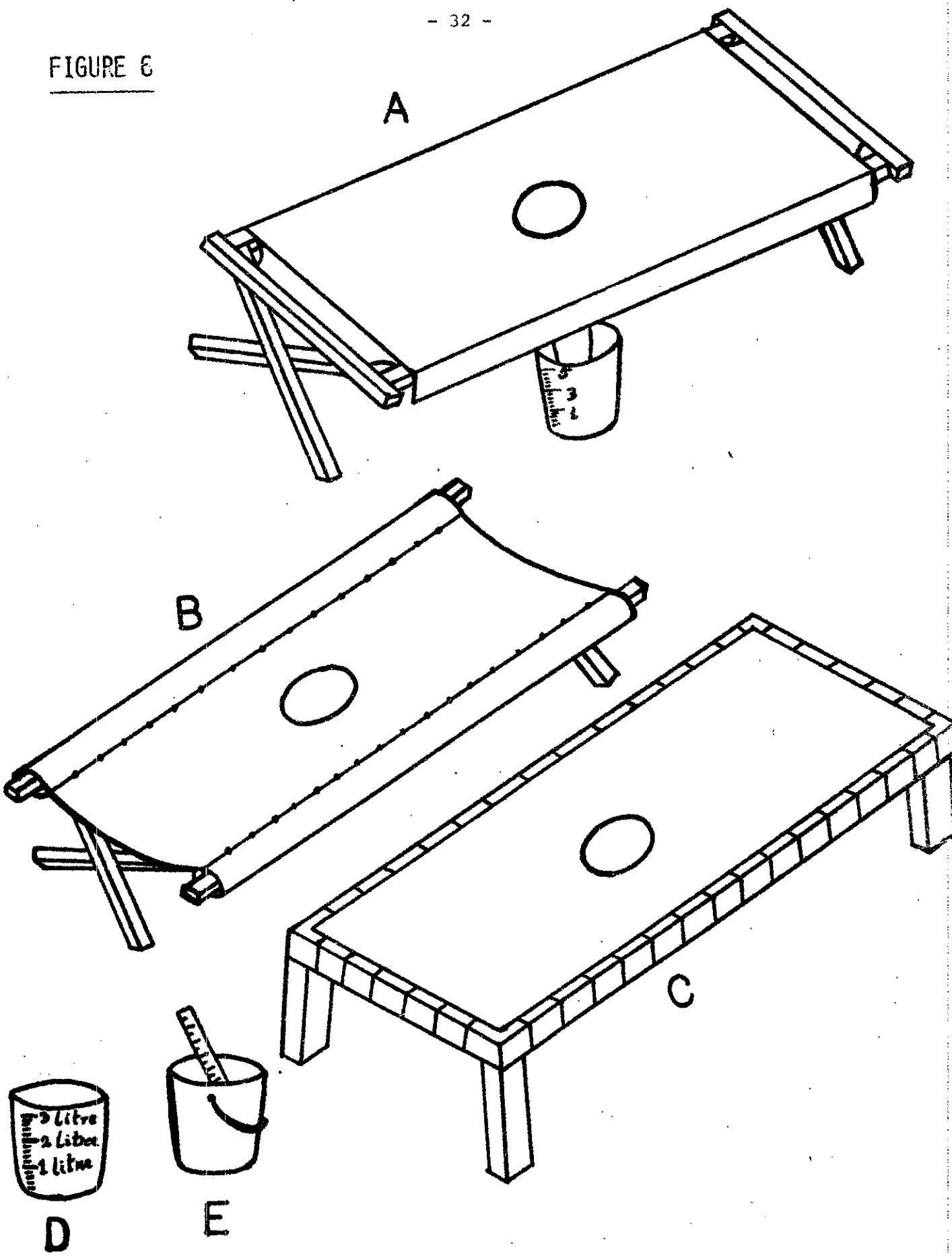
C. Jute on wood frame bed.

D. Calibrate bucket for stool to be placed under cholera cots.

E. Bucket with calibrated stick as alternative method.

The plastic sheet is desirable but not essential for effective use of these cots. The size of the cots can be adjusted for children or adults.

FIGURE 6



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