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No. 52

EXPERT COMMITTEE  
ON CHOLERA

First Report

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WORLD HEALTH ORGANIZATION

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GENEVA

MAY 1952

## EXPERT COMMITTEE ON CHOLERA

### First Session

New Delhi, 19-20 November 1951

#### Members :

- Dr. W. Burrows, Professor of Bacteriology, Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill., USA
- Dr. J. Genevray, ancien Directeur général des Instituts Pasteur d'Indochine, Dijon, France
- Dr. C. C. B. Gilmour, Director, Public Health Laboratory, Memorial Hospital, Peterborough, Northamptonshire, United Kingdom
- Dr. M. A. Gohar Bey, Professor of Bacteriology, Faculty of Medicine, Fouad I University, Cairo, Egypt (*Vice-Chairman*)
- Dr. M. Jafar, Director-General of Health, Karachi, Pakistan
- Dr. C. G. Pandit, Secretary, Indian Council of Medical Research, New Delhi, India (*Chairman*)
- Professor K. Subrahmanyam, Professor of Sanitary Engineering, All-India Institute of Hygiene and Public Health, Calcutta, India

#### Secretariat :

- Dr. R. Pollitzer, Division of Epidemiological Services, WHO (*Secretary*)
- Major-General Sir Sahib Singh Sokhey, Assistant Director-General, Department of Central Technical Services, WHO
- Dr. C. Mani, Director, WHO Regional Office for South-East Asia, New Delhi, India
- Dr. W. Bonne, Acting Director, Division of Communicable Disease Services, WHO
- Dr. S. Swaroop, Chief, Statistical Studies Section, WHO
- R. Pavanello, Public Health Engineer, WHO Regional Office for the Eastern Mediterranean, Alexandria, Egypt

The report on the first session of this committee was originally issued in mimeographed form as document WHO/Cholera/24, 2 January 1952.

## EXPERT COMMITTEE ON CHOLERA

### First Report <sup>1</sup>

In continuation of three sessions held by the Joint OIHP/WHO Study-Group on Cholera—in Paris, 5-7 April and 13-15 October 1948, and in New Delhi, 18-19 November 1949 <sup>2</sup>—the first session of the Expert Committee on Cholera was held on 19 and 20 November 1951 at the WHO Regional Office for South-East Asia, New Delhi, India.

As in 1949, the experts invited by WHO, before holding their own meetings, met in joint session with the Cholera Advisory Committee of the Indian Council of Medical Research on 16 and 17 November.<sup>3</sup> At these joint meetings, which were presided over by Dr. K. C. K. E. Raja, Director-General of Health Services, Government of India, New Delhi, the following subjects, most of which were also on the agenda of the Expert Committee on Cholera, were discussed :

- (1) Technique of examination of stools and water for the presence of *Vibrio cholerae*.
- (2) Further report on the retrospective diagnosis of cholera through the study of the agglutinin response following anti-cholera inoculation.
- (3) Characterization of *V. cholerae*.
- (4) Serological studies on *V. cholerae*.
- (5) Immuno-chemical studies on *V. cholerae*.
- (6) Presence, persistence, and virulence of *V. cholerae* in the stools of convalescents and contacts.
- (7) Epidemiological importance of cholera carriers.

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<sup>1</sup> The Executive Board, at its ninth session, adopted the following resolution :  
The Executive Board

1. NOTES the report of the Expert Committee on Cholera on its first session ;
2. THANKS the members of the committee for their work, and
3. AUTHORIZES publication of the report.

(Resolution EB9.R52, *Off. Rec. World Hlth Org.* 40, 18)

<sup>2</sup> See *Off. Rec. World Hlth Org.* 11, 15; 19, 24 ; *World Hlth Org. techn. Rep. Ser.* 1950, 18.

<sup>3</sup> See Annex 1, page 15, for list of participants.

(8) Possible role of fish and other aquatic animals in the preservation and spread of cholera infection.

(9) Importance of a general programme of environmental sanitation in the campaign against cholera.

At the meetings of the Expert Committee on Cholera, for which Dr. C. G. Pandit was elected Chairman, and Dr. M. A. Gohar Bey, Vice-Chairman, the following subjects were considered.

### 1. Technique of Examination of Stools and Water for the Presence of *V. cholerae*

The Joint OIHP/WHO Study-Group on Cholera, meeting in Paris in October 1948, recommended a comparative study of the results obtained with Bandi's test and with the classical methods of cholera diagnosis respectively.<sup>4</sup>

Work carried out accordingly at the School of Tropical Medicine, Calcutta, in the course of which Bandi's technique as applied by Egyptian workers<sup>5</sup> was compared with cultivation on modified Wilson and Reilly and bile salt agar media, showed that the former method was useful only as long as *V. cholerae* preponderated in the stools, whereas preponderance of coliform organisms considerably affected the results. Consequently in a series of 285 stools, 158 of which were cholera-positive, the culture methods gave 38% more positive results than the examinations according to Bandi's method.

Considering this evidence, the committee came to the conclusion that Bandi's test did not give sufficiently reliable results to recommend its adoption for the laboratory diagnosis of cholera.

The committee recommended, however, that media enriched with potassium tellurite as described by Gohar<sup>6</sup> and the starch-containing medium devised by Dishon<sup>7</sup> should be investigated in parallel tests along with those in current use so as to determine their suitability for the laboratory diagnosis of cholera.

Generally speaking the committee felt that it would be most desirable to find a method which would combine full reliability with rapidity for the isolation of *V. cholerae* at all stages of the infection.

<sup>4</sup> *Off. Rec. World Hlth Org.* 19, 25

<sup>5</sup> See Annex 2, page 16.

<sup>6</sup> See Annex 3, page 17.

<sup>7</sup> Dishon, T. (1951) *Bull. Res. Coun. Israel*, 1, No. 1-2, p. 158

## 2. Agglutinin Response for the Retrospective Diagnosis of Cholera

Considering in 1949 a paper by Krishnan & Dutta on the retrospective diagnosis of cholera through study of the agglutinin response following anticholera inoculation,<sup>8</sup> the Joint OIHP/WHO Study-Group on Cholera came to the conclusion that this method was likely to prove valuable:

(a) as a means of arriving at a retrospective diagnosis in individual cases; and

(b) in assessing the state of immunity in the population of endemic areas.

A continuation of this work was therefore recommended.<sup>9</sup>

From a report presented at the joint meeting of the Cholera Advisory Committee and the Expert Committee on Cholera on 17 November 1951 it would appear that on further investigation the usefulness of such tests for a retrospective diagnosis of cholera in individual cases had not been confirmed. Possibly the method might be helpful in establishing the nature of infection in groups of people who, giving a history of diarrhoea and vomiting, were suspected of having recently suffered from cholera. However, field investigations made on a sufficiently large scale would be required to confirm the validity of this presumption.

Considering this evidence, the committee came to the conclusion that the method thus far had not given results of definite practical value. Should work in this direction be continued, attention ought to be given to the possible role of cross-reactions due to the presence of brucella or salmonella infections. For this reason and on general grounds great attention ought to be paid to the advisability of using in addition vibriocidal tests for the retrospective diagnosis of cholera.

## 3. Serological Studies on *V. cholerae*

As noted above, at the joint meetings of the Cholera Advisory Committee and the Expert Committee on Cholera, consideration of the serology of *V. cholerae* was preceded by a general discussion on the characterization of this micro-organism. The speaker introducing the latter subject challenged the contention of Gardner & Venkatraman<sup>10</sup> that the non-haemolytic vibrio with the specific O antigen of subgroup I represented the only class of vibrios known for certain to cause epidemic cholera. Stating

<sup>8</sup> *World Hlth Org. techn. Rep. Ser.* 1950, **18**, 15

<sup>9</sup> *World Hlth Org. techn. Rep. Ser.* 1950, **18**, 5

<sup>10</sup> Gardner, A. D. & Venkatraman, K. V. (1935) *J. Hyg., Camb.* **35**, 262

that sometimes "non-agglutinable" vibrios alone could be isolated from patients showing clinical features of cholera, he maintained that :

"except for a preconceived notion about the exclusive characterization of *V. cholerae*, no reason could be assigned for the non-acceptance of the non-agglutinable vibrios as causal agents."

In the opinion of the speaker it might also

"be justly inferred that the vibrio is liable to quick changes in nature in respect of the so-called essential properties, namely non-haemolysability and O sub-group I agglutinability".

He reached the conclusion that :

"actually all descendants of a vibrio which has caused cholera in man and is capable of doing so are entitled to that name, irrespective of their size, shape, cultural and biochemical reactions, chemical and antigenic constitution, or pathogenic and epidemic behaviour, unless they have undergone permanent mutation",

and recommended that suitable tracer substances like radioactive isotopes should be incorporated into known cholera vibrios and that their descendants be thereby followed in nature and in the laboratory.

In the course of the prolonged discussion which followed, most speakers expressed disagreement with the views expressed above. Whilst admitting that even during epidemics it was not invariably possible to find the causative organism in the stools of patients showing clinical features of cholera, this was mainly due to extrinsic causes such as unsuitability of the samples submitted for examination or failures in technique. In view of the ubiquity of cholera-like "non-agglutinable" vibrios in surface waters, such micro-organisms could easily be present in the stools of cholera patients without playing a causative role.

Whilst the need was realized for thorough studies on the mutation of *V. cholerae*, it was stressed that the gaps still existing in the knowledge on this subject did not detract from the practical value of the tests adopted for the laboratory diagnosis of the infection. Endorsing this opinion, the committee reached the conclusion that the present definition of the cholera vibrio, though incomplete, was sufficient for practical purposes.

At the same time the committee recognized the need for further studies in this direction, particularly in order to ascertain the desirability of determining the smooth or rough character of the vibrios with sera prepared against rough strains or by other means. In this connexion the committee noted with interest a new method recommended for this purpose.<sup>11</sup>

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<sup>11</sup> See Annex 4, page 18.

The committee also considered it desirable that a study of the antigenic structure and virulence of vibrio strains isolated on successive days of infection from individual cholera patients should be undertaken.

#### **4. Immuno-chemical Studies on *V. cholerae***

Noting a report summarizing the present knowledge on this subject,<sup>12</sup> the committee, while recognizing the need for further immuno-chemical studies on *V. cholerae*, expressed the opinion that when work was being continued in this direction emphasis should be placed on :

(a) studies of cholera endotoxin in relation to the antigenic components of the cholera vibrio ; and

(b) the nature of the enzymic structure of various types of cholera and cholera-like vibrios and the possible bearing of this structure on the pathogenicity and virulence of vibrios.

#### **5. Creation of a Cholera Research Centre**

In the course of the discussions on the mutation of *V. cholerae* during the joint meetings it was pointed out that the Central Research Institute, Kasauli, possessed a considerable collection of different vibrio strains and was prepared to make these available to workers elsewhere. However, no full-time staff was available for studying the collected strains.

The committee stressed the desirability therefore of setting up a cholera research centre for the continuous study of the cholera problem, including the detailed investigation of cholera vibrios from all aspects, employing a team of full-time workers for the purpose. The activities of such a centre could be increased by inviting other workers to participate in the research programme on an exchange basis and by institution of special fellowships.

A beginning could be made by establishing a vibrio reference laboratory with the specific purpose of studying the strains isolated, from the point of view of their epidemiological significance, advantage being taken of phage typing for the purpose.

#### **6. Presence, Persistence, and Virulence of *V. cholerae* in the Stools of Convalescents and Contacts**

The results of a study on the period of excretion of cholera vibrios by convalescents carried out under the auspices of the Office International

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<sup>12</sup> Unpublished working document WHO/Cholera/15

d'Hygiène Publique during the 1950 epidemic in Calcutta<sup>13</sup> were brought to the notice of the committee. These investigations showed that out of 113 convalescents continuously observed in the isolation hospital until five or more successive daily negative cultures were obtained from their stools, 71.6% were negative after the first week, 89.3% after two weeks, and 98.1% after three weeks. The longest period of continuous daily excretion of cholera vibrios was 15 days, the longest period of intermittent excretion 25 days.

In five of the convalescents subsequent examination showed the presence of cholera vibrios belonging to a serological subgroup different from that originally isolated. Obviously these findings were the result of re-infections occurring in the wards. It was considered likely that other instances of re-infection also occurred but could not be proved because they were caused by cholera vibrios of the type originally found in the patients in question. Therefore, it was concluded that "re-infection and cross-infection cannot be ruled out as a cause of prolonged and irregular excretion in hospitals, quarantine camps, and in endemic areas".

Considering these as well as other data available in regard to the presence and persistence of cholera vibrios in the stools of convalescents and contacts, the committee noted that the excretion of such vibrios was as a rule of short duration and intermittent. However, the possibility of longer periods of excretion in a few cases could not be ruled out. The committee concluded that in general the results confirmed the views previously accepted in this respect.

The committee found that so far no conclusive evidence was available as to whether or not and to what extent the vibrios excreted by convalescent and contact carriers tend to be rough and to have an altered virulence. It would be highly desirable to elucidate this point through systematic studies, advantage being taken of the recently recommended serological tests for the recognition of the R-type of *V. cholerae*.

### 7. Epidemiological Importance of Cholera Carriers

The question of whether or not and to what extent convalescent and contact carriers play a role in the spread of cholera was the subject of considerable debate both during the joint meetings and those of the committee.

Those workers with long experience in India or countries farther east maintained that such carriers were of no epidemiological importance. Attention was drawn in this connexion to the important but little-noticed

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<sup>13</sup> Unpublished working document WHO/Cholera/13

observations by Nicholls regarding "Carriers of *V. cholerae* who enter Ceylon from South India".<sup>14</sup>

Nicholls calculated that during the period 1924-33 at least 200 cholera carriers must have arrived in Ceylon during a year of average immigration. It was known on the other hand that during the same period there were only ten occurrences of cholera in the areas to which the majority of the carriers went. Nine of these cholera manifestations were due to the arrival of *incubatory* carriers; the origin of the tenth outbreak could not be elucidated. Nicholls concluded, therefore, that the great majority of the carriers must have been excreting avirulent vibrios.

It was also emphasized that the observations made in West Pakistan since the partition of India fully supported the experiences in Ceylon. Though hosts of immigrants or other travellers, using all available means of transport, arrived in West Pakistan from all parts of India without being subjected to quarantine measures, no importation of cholera took place.

However, it was pointed out to the committee that the observers of the 1947 cholera outbreak in Egypt came to the conclusion that carriers of the infection played a role in that epidemic. The member of the committee advocating this view also went on record that:

"there is no hard and fast line between a contact carrier and a person incubating the disease. The incubation period in some instances is exceptionally long. A contact carrier may come down with the disease, especially after indiscretion in food, or may rid himself of the infection. In both instances the organism is the same, yet the first person became a case, the second remained a carrier for a variable period. Maintaining the view of the non-infectivity of the contact carrier may also imply the non-infectivity of a person in the incubation period or what amounts to a case."

Summing up the discussion on this subject the committee concluded that:

(a) The question as to whether or not or to what extent the vibrios excreted by cholera convalescents and contacts are dangerous from the point of view of a spread of the infection is difficult to answer.

(b) As regards contact carriers the Egyptian workers, as a result of their experience in the epidemic of 1947, consider that contact carriers may play a role in the spread of cholera. The observations made in Ceylon, China, India, and Indochina during many years, on the other hand, do not point to contact carriers as playing a significant role. The trend of opinion in the committee was to the effect that contact carriers do not play a significant role in the spread of the infection.

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<sup>14</sup> Nicholls, L. (1935) *Indian J. med. Res.* **22**, 713

### 8. Possible Role of Fish and Other Aquatic Animals in the Preservation and Spread of Cholera Infection

Discussing the viability of *V. cholerae* outside the human body, the Joint OIHP/WHO Study-Group on Cholera at its 1949 session recommended that :

“ Following the Japanese work on infected fish and the dissemination of cholera through that source, and in view of the possibility that fish infected with cholera vibrios could infect water-supplies, further investigations on the role of fish and other aquatic fauna and their potential danger in infecting water-supplies should be made.”<sup>15</sup>

In a preliminary communication dealing with this subject which was presented to the committee, Pandit & Hora<sup>16</sup> maintained that the hilsa fish (*Hilsa ilisha*) deserved prime attention in this connexion for the following reasons :

(a) A striking similarity was found to exist between maps drawn to show the main foci of cholera endemicity in India and those showing the areas where the main hilsa fisheries were located.

(b) Some correlation seemed to exist between the movements of hilsa during the various seasons of the year and the seasonal variations in cholera incidence.

(c) Apparently there was also some correlation between the five-yearly peaks in hilsa fishery and the periodicity of cholera in Eastern Bengal.

(d) The requirements suitable for a survival of the cholera vibrio in water, namely, (i) a high organic content of the latter, (ii) a suitable concentration of salts, and (iii) an absence of the lethal effects of sunrays, seemed to be fulfilled in the normal environment of hilsa which was migrating near the bottom of the rivers.

(e) The methods of handling hilsa fish to prepare them for consumption are compatible with the assumption that these fish may play a role in the spread of cholera, the procedure being not dissimilar to the dispersal of the vibrios in the environment through faecal matter.

In the prolonged discussions which followed the presentation of Pandit & Hora's thesis, both during the joint meetings and those of the committee, the necessity of further studying the possible role of hilsa fish in causing and maintaining cholera outbreaks was unanimously admitted. At the same time, however, some of the speakers pointed out that part of the evidence adduced by Pandit & Hora in support of their assumption

<sup>15</sup> World Hlth Org. techn. Rep. Ser. 1950, 18, 4

<sup>16</sup> Pandit, C. G. & Hora, S. L. (1951) *Indian J. med. Sci.* 5, 343

could be differently interpreted. In particular it was stated that : (i) the cholera endemicity map was not only similar to the hilsa fishery map but that there was also a remarkably close correspondence between the former and the maps showing the density of population ;<sup>17</sup> (ii) the seasonal appearance of cholera in the inland focus situated along the Ganges in Bihar, ascribed by Pandit & Hora to hilsa migrations, might be actually due to the influx of seasonal labourers.

A discrepancy of opinion existed also regarding the methods best suited for an investigation of the possible role of hilsa fish. Some of the participants in the discussions urged that as an initial step a field laboratory should be set up in one of the cholera-endemic areas where fishing was a normal occupation of the people, in order to examine large numbers of hilsa fish as well as of other aquatic animals caught in nature before, during, and after cholera outbreaks. Pandit & Hora, on the other hand, advocated that laboratory studies should be attempted in the first instance. The committee, recognizing the need for a study of the role of fish and other aquatic animals in the spread of cholera, considered it desirable that an investigation planned for this purpose should be sufficiently broad-based to include field investigations as well as laboratory studies.

#### **9. Importance of a General Programme of Environmental Sanitation in the Campaign against Cholera**

A report assessing the possibilities of implementing programmes for environmental sanitation in cholera-endemic areas, particularly in rural localities,<sup>18</sup> was presented to the committee. The important point was stressed that instead of insisting on elaborate programmes, the cost of which was usually prohibitive, less ambitious schemes adapted to the financial resources, habits, and the level of health consciousness of the people should be applied. Evidence was adduced to show that the implementation of such programmes was not only apt materially to reduce the cholera incidence but proved also of outstanding value by stimulating the interest of the people in the work and inducing them to co-operate.

Considering this report and realizing that good sanitation is the most effective method of cholera prevention, the committee urged that all authorities concerned should continue studying possibilities for the improvement of environmental sanitation in areas where cholera is endemic, particularly the use of methods applicable with local materials and at a

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<sup>17</sup> Unpublished working document WHO/Cholera/17

<sup>18</sup> Unpublished working document WHO/Cholera/12

cost within the economic possibilities of the areas concerned. Health education should form an integral part of the programmes contemplated.

In the opinion of the committee even partial programmes, if persisted in for long periods, will yield good results by stimulating the interest of the people to such a degree that they will be ready to shoulder the entire outlay for the work and will demand an extension of the programme. The committee stressed the urgency of initiating environmental sanitation programmes in both urban and rural localities where cholera is endemic.

#### **10. Action of the Expert Committee on Biological Standardization Regarding Cholera Vaccines and Diagnostic Sera**

Considering a report by the Secretary of the Expert Committee on Biological Standardization,<sup>19</sup> the committee was in general in hearty accord with the action taken by that committee in regard to cholera but wished to emphasize the following points :

(a) The use of a standard homogeneous strain of mice is essential for permitting a comparison of the results obtained by different laboratories. Every effort should be made to maintain and to make generally available the strain of Swiss mice of the Yellow Fever Laboratories of the International Health Division of the Rockefeller Foundation, New York, that has been suggested, or some other equally susceptible strain that may be selected subsequently.

(b) In the opinion of the committee the suggested use of freeze-dried antisera is open to considerable question in view of repeated observations on the irregular destruction of antibodies in the process.

#### **11. Discussion of a Memorandum from the Director-General to the Expert Advisory Panel on Cholera in Regard to the Provisions Relating to Cholera in WHO Regulations No. 2**

In accordance with a resolution of the Executive Board at its eighth session,<sup>20</sup> a memorandum<sup>21</sup> on the provisions relating to cholera in the International Sanitary Regulations (WHO Regulations No. 2)<sup>22</sup> was

<sup>19</sup> Unpublished working document WHO/Cholera/20

<sup>20</sup> Resolution EB8.R13, *Off. Rec. World Hlth Org.* 36, 4

<sup>21</sup> Unpublished working document WHO/Epid/57—WHO/Cholera/10

<sup>22</sup> *World Hlth Org. techn. Rep. Ser.* 1951, 41

presented to the committee for comment and recommendations. In this document it was pointed out that in framing these regulations a compromise on certain points had to be reached and that for this reason the text does not always attain the ideal of technical perfection. It was recommended that any technical criticisms of, or proposed amendments to, the Regulations should be made in the full knowledge of the difficulties encountered in reaching an agreement regarding some points.

Bearing these considerations in mind, the committee did not wish to make far-reaching recommendations. It was noted that, the Expert Committee on Biological Standardization finding it impossible pending further investigations to arrive at an adequate standard for cholera vaccines,<sup>23</sup> the Fourth World Health Assembly had decided not to state the dosages of such vaccines in the form for the international certificate of vaccination or revaccination against cholera.<sup>24</sup>

## 12. Cholera Field-Work

### 12.1 *Draft plan for cholera field-studies in East Pakistan*

A draft plan for cholera field-studies in East Pakistan which had been distributed among the members of the committee envisaged activities in three endemic areas, each with a population of 30,000. It was proposed to carry out sanitary improvements in one of these areas, wholesale inoculation before the onset of the cholera season in the second, whilst the third was to serve as a control area where, should cholera appear, only the routine programme of control measures would be implemented by the local health authorities.

The committee was informed, however, that it is now proposed to limit the project to a study of the possibilities of controlling cholera in an endemic area through implementation of sanitary improvements and of health education. Desirable though it would be to study the influence of cholera vaccination as well, this ought to be the object of a special project.

Noting these proposals, the committee hoped that the programme for sanitary improvements would be adapted to the local conditions and in particular to the economic level of the study area.

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<sup>23</sup> See *Off. Rec. World Hlth Org.* 8, 7; 11, 8; *World Hlth Org. techn. Rep. Ser.* 1950, 2, 4; 1951, 36, 3.

<sup>24</sup> Resolution WHA4.75, *Off. Rec. World Hlth Org.* 35, 50; see also *Off. Rec. World Hlth Org.* 37, 128.

### 12.2 *Studies on cholera endemicity*

The committee was informed that statistical studies on the problem of cholera endemicity now in progress have shown the great need for delimiting the endemic foci within the various cholera-affected countries through statistical analysis of the situation in the smallest units of population in regard to which data are available or can be elicited through further field-studies. The important fact has been brought out that the situation is by no means uniform throughout the so-called endemic areas as known at present, but that marked differences may exist even between quite small adjacent subdistricts. No doubt studies to elucidate the reasons underlying such differences might shed light upon the still incomplete knowledge of cholera epidemiology. At the same time an exact demarcation of the real endemic foci, which quite probably are often more limited in extent than is usually assumed, would be of great value for the institution of truly rational control measures.

### 12.3 *Cholera vaccination campaigns with special reference to the new casein hydrolysate vaccine*

Noting the outstanding qualities of Sokhey's biologically standardized casein hydrolysate vaccine,<sup>25</sup> the committee recommended large-scale field-studies so as to determine whether the results of vaccination in man corroborate the most encouraging results obtained in experimental animals.

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<sup>25</sup> Sokhey, S. S. (1950) *Bull. World Hlth Org.* 3, 33

**Annex 1****PERSONS PRESENT AT THE JOINT MEETINGS  
OF THE WHO EXPERT COMMITTEE ON CHOLERA,  
AND THE CHOLERA ADVISORY COMMITTEE OF THE  
INDIAN COUNCIL OF MEDICAL RESEARCH***Members of the Cholera Advisory Committee :*

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Shri S. P. Jain, Statistician, Labour Bureau, Ministry of Labour,  
Government of India, Simla  
Dr. K. V. Krishnan, Professor of Microbiology, All-India Institute  
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Dr. R. B. Lal, Professor of Epidemiology, All-India Institute of Hygiene  
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Dr. Y. S. Narayana Rao, Director, King Institute, Guindy, Madras  
Dr. K. V. Venkatraman, Serologist and Chemical Examiner to the  
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Dr. P. M. Wagle, Director, Haffkine Institute, Bombay

*Other participants :*

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Office for South-East Asia, New Delhi  
Dr. S. L. Hora, Director, Zoological Survey of India, Calcutta  
Lt-Col. C. L. Pasricha, Medical Adviser to the High Commissioner  
for India, London, United Kingdom  
Dr. K. C. K. E. Raja, Director-General of Health Services, Govern-  
ment of India (*Chairman*)  
Dr. C. V. Ramchandani, Assistant Director-General of Health Services,  
Government of India

Dr. D. L. Shrivastava, Assistant Director (Planning), Central Drug Research Institute, Lucknow

Dr. Gurkirpal Singh, Assistant Director, Central Research Institute, Kasauli

Dr. R. L. Tuli, Regional Public Health Adviser, WHO Regional Office for South-East Asia, New Delhi

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## Annex 2

### TECHNIQUE OF BANDI'S TEST<sup>1</sup>

To a Bandi tube containing 15 ml of peptone water of pH 8.2 an amount of high-titre cholera O serum (Inaba and Ogawa) sufficient to bring the final dilution of the serum in the medium to about 50% of the original titre was added. Suspected cholera stool was added and examination was made hourly during 3-7 hours' incubation. In positive cases granules due to an agglutination of the vibrios would appear in the body of the fluid and also on the sides of the tube. These granules were stained and examined microscopically for confirmation. Two controls were always made, one by inoculating the material in a tube containing peptone water only, the other by inoculating known cholera vibrios into a tube containing serum.

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<sup>1</sup> Note submitted by Dr. S. C. Ghosal and B. M. Paul, School of Tropical Medicine, Calcutta, India.

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### Annex 3

## MEDIA ENRICHED WITH POTASSIUM TELLURITE FOR THE LABORATORY DIAGNOSIS OF CHOLERA<sup>1</sup>

### Preparation of the Media

To ordinary sterile peptone water 0.2% exsiccated sodium carbonate and 0.5% sodium taurocholate are added. After distribution into 25-ml conical flasks which are filled to the bottom of the neck, sets of three flasks are prepared for each specimen to be examined by dissolving potassium tellurite to a concentration of 1/100,000 in the first flask, to a concentration of 1/200,000 in the second flask, and to a concentration of 1/400,000 in the third. The final pH is about 9.

If it is necessary to be economical, it is permissible to use only one flask or even a tube containing 10 ml of the medium, adding potassium tellurite to a concentration of 1/200,000.

To facilitate preparation, sterile stock solutions containing 10% sodium carbonate, 10% sodium taurocholate, and 1% potassium tellurite respectively are kept on hand. Sterilization may be done in a steam sterilizer.

### Technique of Examination

The flasks are heavily inoculated with the stools and incubated at 37°C. After 8 and 24 hours, loopfuls are taken from the surface of the media with the aid of a wire bent at right angles, and plated out on alkaline agar. After incubation of the plates at 37°C for 18-24 hours, suspicious colonies are picked out and are used for inoculation of the butt as well as of the slope of semi-sloped agar containing 1% mannite, 0.1% glucose, and Andrade's indicator. Glucose may be dispensed with.

### Growth Appearances

Cholera vibrios produce in the semi-sloped medium a red colour in the butt and a pink tinge on the sloped part of the medium. The colour reactions produced by *Escherichia coli* are the same, but gas is produced as well.

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<sup>1</sup> Note submitted by Dr. M. A. Gohar Bey, Professor of Bacteriology, Faculty of Medicine, Fouad I University, Cairo, Egypt.

*Bacillus faecalis alcaligenes* leaves the medium unaltered while, if glucose is present, most *Proteus* strains turn the butt only red.

Final identification of suspicious growths is carried out by agglutination tests with cholera O antiserum.

#### Examination of Water Samples

One or more litre flasks are filled to the bottom of the neck with the water to be examined. The water is then transformed into a selective medium by the addition of 1% peptone, 0.5% sodium chloride, 0.2% sodium carbonate, 0.5% sodium taurocholate, and potassium tellurite to a concentration of 1/200,000.

The flasks are incubated at 37°C. Loopfuls are taken after 8 and 24 hours from the surface with the aid of a bent wire and the procedure outlined in the case of stool examination is followed.

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#### Annex 4

#### A NEW TEST FOR THE IDENTIFICATION OF ROUGHNESS IN V. CHOLERAE<sup>1</sup>

To demonstrate the selective action of guinea-pig complement on rough vibrios, the following technique is recommended :

An 18 hours' peptone-water culture of the strain to be tested is diluted 100-fold with peptone water. One part of this dilution is mixed with two parts of complement diluted with an equal amount of peptone water. The mixture is incubated for four hours at 37°C. The initial inoculum and the subsequent growth after 4 hours' incubation at 37°C are sampled by planting 3-mm loops on nutrient agar plates without spreading. Results are read after 18 hours' incubation at 37°C. The difference between the cultures which have been affected by the complement and those which have not is so marked as to be easily seen by the naked eye. A control without the complement is included in the test. It must, however, be pointed out that not all guinea-pig sera are suitable for use in the test. Preliminary tests with known smooth strains must be carried out so as to exclude guinea-pig sera exerting inhibitory or bactericidal action on smooth cholera vibrios.

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<sup>1</sup> Note submitted by Colonel M. L. Ahuja, Director, Central Research Institute, Kasauli, India.

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