Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1963, **28**, 379-383

Protective Activity of Cholera Vaccines against El Tor Cholera Vibrios

MARGARET PITTMAN, Ph.D.[1] & JOHN C. FEELEY, Ph.D.[2]

With the advent of epidemic El Tor cholera there has been concern about the value of cholera vaccine prepared from classical cholera strains.

Using a recently developed mouse protection test, the authors have determined the protective activity of five commercial vaccines against strains of epidemic El Tor and classical cholera vibrios. Both Ogawa and Inaba types of each kind of vibrio were used. With each vaccine, protection against El Tor vibrios was as good as that against classical cholera vibrios. There was, however, a greater than tenfold difference in the potency of the vaccines.

The findings support the use of cholera vaccine for immunization against El Tor cholera and show that there are wide variations in the mouse-measured potency of lots of vaccine. The significance of the findings must await results of controlled field trials.

The recent epidemics of El Tor cholera in Hong Kong (MacKenzie, 1961), the Philippines, and other South-East Asian areas have been of particular interest not only from the standpoint of international quarantine (*Wkly. epidem. Rec.*, 1962) but also to see whether the vaccines prepared from classical cholera vibrio strains would provide protection against El Tor cholera. Mukerjee & Guha Roy (1962) have suggested that this vaccine should be regarded as of doubtful value for mass immunization against outbreaks of El Tor cholera. This supposition was based on the chemical and antigenic differences between El Tor and classical cholera strains (Linton, 1940) and one brief report of failure to obtain satisfactory cross-protection in animals.[3]

A number of differences between the two vibrios have been described: haemolytic activity, toxin, protein composition, sublimate precipitation, soda agglutination, and heat and chloroform inactivation of agglutinability (see Pollitzer, 1959). Bacteriophage typing (Mukerjee, 1961) and haemolysin testing[4] seem to provide concise methods for differentiating the two vibrios. In the past, haemolysin detection has been capricious (de Moor, 1949; Tanamal, 1959). In our experience this was especially true when the haemolysis test was performed using three-day-old cultures according to the method of Greig (1914).

In spite of differences, *Vibrio cholerae*[5] and the El Tor vibrio[6] have common characteristics. They belong to the Heiberg fermentation group I, and have common O group antigens. In this paper it will be shown that "classical" cholera vaccines are capable of protecting mice against El Tor strains to the same degree as against classical cholera strains.

MATERIALS AND METHODS

Mouse protection test

The test employed conforms in principle to a test proposed by the WHO Study Group on Requirements for Cholera Vaccine (1959). Our test was

[1] Chief, Laboratory of Bacterial Products, Division of Biologics Standards, National Institutes of Health, Public Health Service, US Department of Health, Education, and Welfare, Bethesda, Md., USA.

[2] Laboratory of Bacterial Products, Division of Biologics Standards, National Institutes of Health, Public Health Service, US Department of Health, Education, and Welfare, Bethesda, Md., USA.

[3] Tanamal, S. J. W., Watanabe, Y. & Felsenfeld, O. (1958) Paper delivered to the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Miami, Fla., 8 November 1958 (unpublished).

[4] See the article by Feeley & Pittman on page 347 of this issue.

[5] *V. comma* in *Bergey's Manual of Determinative Bacteriology*, 7th ed. (Breed et al., 1957).

[6] Hugh (1962) has suggested the designation *Vibrio comma*, El Tor biotype.

TABLE 1

SOURCE AND CHARACTERISTICS OF CHALLENGE STRAINS [a]

Cholera strain	Source	Subtype	Haemolysin	Average LD_{50} (colony-forming units)
NIH 41 (classical)	Calcutta, India, received from Walter Reed Army Institute of Research, 1941.	Ogawa	—	7 (19) [b]
NIH 35A3 (classical)	Kasauli, India, received as above, 1942.	Inaba	—	83 (19) [b]
HK-1 (El Tor)	Hong Kong, 1961, from Dr D. J. M. MacKenzie.	Ogawa	+	<1 (3) [b]
V-86 (El Tor)	Hong Kong, 1961, from Dr D. J. M. MacKenzie.	Inaba	+	<1 (3) [b]
Manila 30810 (El Tor)	Philippines, 1961, isolated by Dr H. L. Smith, Jr and author (J. C. F.).	Ogawa	+	<1 (3) [b]

[a] All strains fermented sucrose and mannose but not arabinose (Heiberg Group I).

[b] The number of virulence tests is shown in parentheses. Vibrios were suspended in 5% mucin and inoculated intraperitoneally.

outlined in a preliminary communication made in 1960 and now in press (Feeley & Pittman, 1962). Briefly, mice in three groups of 16 each were given one intraperitoneal injection of three graded doses of vaccine, respectively, and two weeks later were challenged intraperitoneally with approximately 1000 LD_{50} of culture suspended in 5% hog gastric mucin. In each experiment a monotype reference vaccine was included. Two tests of each lot were performed on separate days, the results were combined, and the potency was expressed relative to the reference, i.e., the ED_{50} of the reference was divided by the ED_{50} of the vaccine. [1]

Challenge vibrio strains

The source and characteristics of the strains are given in Table 1. The two classical cholera strains have been in use for many years in the USA for the preparation and potency assay of cholera vaccine. The three El Tor strains are representative of the strains isolated during the recent epidemics. Strain V-86, however, was the only Inaba strain among 129 isolated in Hong Kong (MacKenzie, 1961). Very few Inaba strains were isolated during the Philippine epidemic. The cultures were freeze-dried promptly after isolation. Identifying characteristics were determined by the methods described by Burrows & Pollitzer (1958).

Vaccines

Five lots of US commercial cholera vaccine designated by code numbers were used. They were selected to represent the variations in potency that have been encountered using the experimental protection test. All lots met the official required potency.

The monotype reference vaccines are freeze-dried preparations and have been used in the Division of Biologics Standards of the National Institutes of Health for experimental work only. Their potencies are comparable to the median values of US commercial vaccines as determined by the test described here. Before drying, the Ogawa vaccine was about 8 times, and the Inaba about 34 times, more potent than the corresponding international reference vaccines (Feeley & Pittman, 1962). On drying, the Ogawa decreased about 25% in potency and the Inaba about 50%. The low values of the international references may be due to loss of protective activity during the process of drying. We observed more than 10-fold losses in early attempts to dry the reference vaccines.

[1] A detailed paper dealing with the mouse protection test is in preparation.

TABLE 2

PROTECTIVE ACTIVITY OF COMMERCIAL CHOLERA VACCINES AGAINST EL TOR AND CLASSICAL CHOLERA VIBRIOS

Challenge strain [a]		Vaccine code No.	ED_{50} (ml)	(SD) [b] (%)	Relative potency (Ref. = 1.0)
Ogawa	HK-1 (El Tor)	21	0.000267	(73-136)	1.31
		11	0.00263	(69-146)	0.133
		Ogawa reference	0.000351	(63-159)	
	NIH 41 (classical)	21	0.000293	(76-132)	0.857
		Ogawa reference	0.000251	(72-139)	
		11	0.000879	(73-136)	0.140
		Ogawa reference	0.000123	(68-147)	
	Manila 30810 (El Tor)	32	0.0000228	(76-132)	10.3
		13	0.000938	(81-124)	0.251
		42	0.000123	(73-137)	1.91
		Ogawa reference	0.000235	(69-146)	
	NIH 41 (classical)	32	0.0000427	(78-128)	4.40
		13	0.000811	(73-136)	0.232
		Ogawa reference	0.000188	(75-134)	
		42	0.000228	(73-136)	1.33
		Ogawa reference	0.000304	(78-128)	
Inaba	V 86 (El Tor)	21	0.000363	(77-130)	1.85
		11	0.00280	(79-127)	0.239
		Inaba reference	0.000670	(72-140)	
	NIH 35A3 (classical)	21	0.000314	(80-124)	1.18
		Inaba reference	0.000369	(69-146)	
		11	0.00121	(77-130)	0.255
		Inaba reference	0.000309	(74-134)	

[a] Challenge doses contained approximately 1000 LD_{50}.

[b] ED_{50} values are the combined results of two tests. Figures in parentheses represent the limits of one standard deviation (SD).

EXPERIMENTAL RESULTS

The five lots of cholera vaccine were tested for protective activity using the Ogawa strains for challenge, and two using the Inaba strains. It may be seen in Table 2 that with either subtype the protective activity of a particular lot of vaccine against the El Tor challenge was as good as against the classical cholera challenge. With the Ogawa strains and No. 11 and No. 13 vaccines, the relative potency values were practically identical. The same was true with Inaba strains and No. 11 vaccine. With the other three vaccines the differences, only 1.4, 1.5

and 2.3 times greater for the Ogawa strains and 1.6 for the Inaba strain, are not statistically significant. It is of interest to note that, in each case where there was a difference, the highest value was obtained with the El Tor challenge.

The wide differences in protective activity of the vaccines are shown in Table 2. Two were comparable to the reference, two were one-fourth or less, and the remaining one was probably more than four times more potent.

No commercial vaccines prepared with El Tor strains were available for converse cross-protection tests. One El Tor vaccine, prepared in the Philippines and received through the courtesy of Dr T. P. Pesigan, was approximately three times more potent than the Ogawa reference (classical cholera challenge); and a fractionated El Tor vibrio antigen from Dr Y. Watanabe and Dr W. F. Verwey, University of Texas Medical Branch, afforded excellent protection against classical cholera challenge (Ogawa). We did not determine the protective activity of either antigen using El Tor for challenge. Watanabe & Verwey (1962), however, reported highly active protection for their antigen against both El Tor and classical vibrios (Ogawa).

DISCUSSION

It has been shown in this report that vaccines prepared from classical vibrios were capable of protecting mice against El Tor vibrios to the same degree as against classical cholera vibrios. Since the details of the experiments by Tanamal et al. (1958) were not given by those authors, we can only hazard a suggestion that their failure to obtain satisfactory protection may have been due to the use of a toxic challenge. Their vaccines were effective against El Tor challenge when El Tor toxoid was added to the vaccine. In our experiments non-toxic challenge doses of the culture, suspended in mucin, were employed.

The role of the toxin of the El Tor vibrio in human infection and of a toxoid in the vaccine is not known. Clinically and pathologically, El Tor and "classical" cholera are indistinguishable and recovery from each infection following fluid and electrolyte therapy is equally dramatic (*Wkly epidem. Rec.*, 1962). It might also be argued that, if infection can be prevented by immunization, the vibrios would not have an opportunity to multiply and liberate toxin, hence toxoid in the vaccine would be of no value. This situation occurs in the prevention of pertussis. *Bordetella pertussis* produces an antigenic toxin but the toxoid is not effective in preventing whooping-cough and vaccine without toxoid is effective.

The results presented also show that US commercial vaccines vary widely in potency as measured by the mouse protection test employed. We have observed, likewise, wide variations in potency in vaccines prepared in other countries (Feeley & Pittman, 1962). Some were even lower in potency but none was as high as the highest example given. Each lot of vaccine used in our study had met the current potency requirement which is determined by vaccinating mice with a single dose of vaccine and challenging them with graded doses of culture (US Public Health Service, 1945). A revision of the official potency test is now under consideration.

Although it has been shown that with the use of a mouse protection test there are large differences in protective activity of cholera vaccines, it remains to be determined if the differences measured in the laboratory are correlated with protective activity in man. In fact, it remains to be proven in adequately controlled field trials that cholera vaccine is of prophylactic value to man.

The evidence presented in this paper does not substantiate the doubts of Mukerjee & Guha Roy (1962) concerning the value of routine cholera vaccine in immunization against El Tor cholera. On the other hand, this report on the effectiveness of cholera vaccine against El Tor infection has been limited to the mouse. Nevertheless, there seems to be some justification for the continued use of commercial "classical" cholera vaccine.

RÉSUMÉ

Les épidémies récentes survenues à Hong-Kong, aux Philippines, et en Indonésie, dues au vibrion El Tor, ont fait poser la question de la valeur protectrice des vaccins anticholériques usuels contre ce type particulier de vibrion Certains chercheurs, s'appuyant sur des différences chimiques et antigéniques, ont exprimé des doutes sur l'efficacité de ces vaccins.

Les auteurs ont utilisé un test de protection quantitative de la souris et ont pu ainsi montrer que: 1) les vaccins américains actuellement dans le commerce protègent la

souris des souches de vibrion El Tor récemment isolées à Hong-Kong et à Manille; 2) le degré de protection est identique à celui fourni contre le vibrion cholérique classique; 3) ces vaccins présentent des différences marquées en ce qui concerne leur efficacité.

Il ne s'agit certes là que d'une expérimentation animale que des essais cliniques contrôlés devront confirmer. En attendant la réalisation de ces essais, il paraît justifié de continuer à se servir des vaccins anticholériques dits classiques.

REFERENCES

Breed, R. S. et al. (1957) *Bergey's manual of determinative bacteriology*, 7th ed., Baltimore, Williams and Wilkins

Burrows, W. & Pollitzer, R. (1958) *Bull. Wld Hlth Org.*, **18**, 275

Feeley, J. C. & Pittman, M. (1962) In: *Proceedings of the SEATO Conference on Cholera, Dacca... 1960* (in press)

Greig, E. D. W. (1914) *Indian J. med. Res.*, **2**, 623

Hugh, R. (1962) *Bact. Proc.*, p. 52

Linton, R. W. (1940) *Bact. Rev.*, **4**, 261

MacKenzie, D. J. M. (1961) *Report on the outbreak of cholera in Hong Kong*, Hong Kong, Government Press

Moor, C. E. de (1949) *Bull. Wld Hlth Org.*, **2**, 5

Mukerjee, S. (1961) *J. Hyg. (Lond.)*, **59**, 109

Mukerjee, S. & Guha Roy, U. K. (1962) *Brit. med. J.*, **1**, 685

Pollitzer, R. (1959) *Cholera*, Geneva (*World Health Organization: Monograph Series*, No. 43)

Tanamal, S. J. W. (1959) *Amer. J. trop. Med. Hyg.*, **8**, 72

US Public Health Service (1945) *Minimum requirements: cholera vaccine*, National Institutes of Health, Division of Biologics Standards, Bethesda, Md.

Watanabe, Y. & Verwey, W. F. (1962) *Bact. Proc.*, p. 89

Wkly epidem. Rec., 1962, **37**, 253

World Health Organization, Study Group... on Requirements for Cholera Vaccine (1959) *Wld Hlth Org. techn. Rep. Ser.*, **179**, 43