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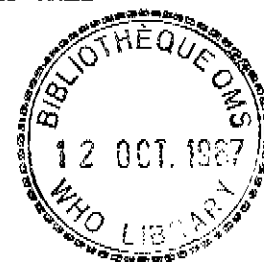
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LABORATORY STUDIES ON CHOLERA VACCINES
USED IN THE 1965 CALCUTTA FIELD TRIAL

A collaborative study¹



In a previous collaborative study of potency assays of the 1964 Calcutta field trial cholera vaccines, a comparison was made only of the outcome of two mouse protection tests (Sokney-Habbu and Feeley-Pittman) with different experimental design. Because of the incomplete block design and failure of laboratories to complete and report their results promptly, only a preliminary report covering tests in two laboratories on the same two vaccines has been issued (See Spaun, WHO/BS/755.65). These results showed that the two mouse protection test methods gave contradictory estimates of relative potency, with the Haffkine vaccine appearing more potent by the Sokney-Habbu method and the Walter Reed vaccine more potent by the Feeley-Pittman method. It is hoped that a final report can be issued soon which will provide data on all vaccines tested, but in view of the low statistical significance of the field trial results, the possibility of any meaningful comparison with the mouse protection data is remote for the 1964 Calcutta vaccines. A report covering the mouse protection assays of the Division of Biologics Standards (DBS) on all four 1964 Calcutta vaccines and the two fluid vaccines (classical and El Tor) employed in the 1964 Philippines field trial was submitted to the Expert Committee on Biological Standardization (see Feeley & Pittman, WHO/BS/774.65). The two fluid vaccines behaved in a similar manner in the field trial, with the El Tor vaccine appearing slightly more protective. The El Tor vaccine appeared slightly, but insignificantly, more protective in the DBS mouse protection tests.

¹ Report prepared by J. C. Feeley, WHO Consultant (Permanent address: Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland) after consultation with participants in the study listed on page 10.

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At the informal meeting of interested laboratory workers held in Honolulu on 28 January 1965, it was agreed that the vaccines employed in the 1965 Calcutta field trial would be evaluated by participating laboratories by a variety of techniques, the choice of which was to be dictated by the experience and interest of the participants. The present report summarizes data available at the time of the writer's visit to the participating laboratories in April 1966.¹

Not all the laboratories which agreed to participate were able to carry out the studies planned in Honolulu, but considerable data were obtained. One difficulty for many laboratories was the enormous task of studying all four vaccines. Another problem in trying to relate these findings to previous field trials is the lack of a common reference vaccine. In Honolulu, it was recommended that the freeze-dried Walter Reed vaccine used in Calcutta (1964) be employed as a reference, but this proved unavailable. Detailed results of their studies were presented in reports submitted by Dr Feeley, Dr Joo, Dr Dutta and Dr Gallut. These reports may be available from the participants. Detailed descriptions of the preparation of each vaccine provided by the manufacturers were already sent to each participant by WHO. The characteristics of the vaccines employed in the field trial are summarized as follows:

1. Kasauli vaccine (Code "X") - agar grown, phenol killed and preserved, manufactured from local Indian Ogawa and Inaba strains of classic V. cholerae.
2. Haffkine vaccine (Code "Z") - grown in casein hydrolysate broth, killed with formalin, preserved with phenylmercuric nitrate, manufactured from local Indian Ogawa and Inaba strains of classic V. cholerae.
3. Philippine El Tor vaccine (Code "G") - agar grown, heat-killed, phenol preserved, manufactured from Philippine Ogawa and Inaba strains of V. cholerae biotype eltor.
4. Walter Reed Army Institute of Research (WRAIR) vaccine (Code "J" or "N") - agar grown, formalin killed, freeze-dried, preserved with phenol after reconstitution, manufactured from U.S. Ogawa and Inaba vaccine strains of classic V. cholerae. Contains approximately 3.5 times the bacterial content of WRAIR vaccine employed in 1964 Calcutta

¹ For list of participants, see p. 10.

field trial. Vaccine J-1, which was distributed to some laboratories by WRAIR was prepared by rehydrating 50-ml bottles of vaccine J, and refreeze-drying in 5 ml amounts. Since vaccine J-1 was not actually used in the field trial, it was omitted from this report.

RESULTS OF LABORATORY STUDIES

1. Chemical data - Nitrogen Content

Table 1 shows the results of nitrogen determinations on the vaccines as performed by the DBS and the "Human" Institute for Serobacteriological Production and Research. Values for nondialyzable nitrogen and acetone precipitated nitrogen are felt to reflect more accurately the actual bacterial content of the vaccine, since total nitrogen values on whole vaccine are greatly influenced by constituents derived from culture media. This is particularly true for the Haffkine vaccine, which contains the casein hydrolysate broth in which the organisms are grown. It would appear that the amount of macromolecular material-associated nitrogen is highest with the WRAIR and CRL¹ vaccines.

2. Toxicity tests on vaccines

Table 2 gives the results of weight-change toxicity tests performed in mice injected intraperitoneally with one-half or one-quarter of a single human dose of the vaccines as employed in the field trials. The WRAIR vaccine is more toxic for mice based on weight changes than the other three Calcutta field trial vaccines which were similar to one another in mouse toxicity. The WRAIR vaccine was only slightly and probably insignificantly less toxic than the CRL vaccine at the test level of one-half the single human dose. The higher toxicities of the WRAIR and CRL vaccines are probably related to their higher bacterial content, since the weight loss in mice is believed to be chiefly a function of gram-negative bacterial endotoxin content.

¹ CRL - vaccine used in Pakistan-SEATO Cholera Research Laboratory (CRL) field trial.

3. Results of mouse protection tests

Data derived from mouse protection tests performed by the Division of Biologics Standards using the Feeley-Pittman method (see Annex III, WHO/ED/Ch.2) are given in Table 3. The relative potencies of the Calcutta field trial vaccines expressed in terms of the respective U.S. Ogawa and Inaba monovalent reference vaccines and the CRL vaccine are shown in Table 4. Potency estimates relative to the CRL vaccine are given both on a per millilitre basis and on the basis of the 0.4 ml human dose employed in the CRL field trials. Relative to both U.S. reference vaccines, the Calcutta vaccines are ranked in the following order of decreasing potency: WRAIR, Kasauli, Haffkine, and Philippine. The WRAIR vaccine was significantly more potent than the other three vaccines, except in the case of Inaba serotype challenge, where its difference from the Kasauli vaccine is not quite significant at the five per cent. level. Differences in the relative potency estimates for Kasauli, Haffkine, and Philippine vaccines are generally not statistically significant at the five per cent. level, although the difference between the Kasauli and Philippine vaccines is of borderline significance for Ogawa challenge.

It is of interest to note that, relative to the CRL vaccine, the WRAIR vaccine was slightly but not significantly more potent on a human dose basis, while the other vaccines are not dramatically less potent; only the Haffkine and Philippine vaccines were significantly less potent. Of course, the vaccines relative to the CRL vaccine are less potent by a factor of 2.5 if potencies are compared on a per millilitre basis.

Mouse protection data submitted by the "Human" Institute for Serobacteriological Production and Research are summarized in Table 5 (for details of individual tests, see Dr Joo's report). The Philippine vaccine was not tested. The WRAIR and Kasauli vaccines appeared essentially similar in potency and both were more potent than Haffkine vaccine.

The DES data were recalculated in terms of Haffkine vaccine for comparison with results from the "Human" Institute and the results are shown in Table 6. Results from both laboratories would rank the vaccines tested in common in the same order of relative potency.

The Central Research Institute, Kasauli, has provided the results of a single test for each serotype challenge in which Kasauli and WRAIR vaccines were tested concurrently, and this is shown in Table 7. They found these vaccines similar in potency against Ogawa challenge, but unlike the results from DBS and "Human" Institute, they found Kasauli vaccine much more potent than WRAIR vaccine against Inaba challenge.

Finally, the Chiba Serum Institute provided data based on duplicate tests for each serotype challenge by which to compare the Philippine El Tor vaccines which were employed in the Calcutta and Philippine field trials respectively. El Tor strains were used for challenge. The vaccines were not significantly different in potency against Inaba challenge, but the Calcutta vaccine appeared approximately one-half as potent (significance of difference is borderline) against Ogawa challenge. It is perhaps worth noting that, although these two Philippine vaccines were not compared directly, DBS tests for these vaccines (using classical *V. cholerae* challenge) yielded potency ratios of 1.3 and 1.0 times U.S. Ogawa Reference, and 3.9 and 3.1 times U.S. Inaba Reference, for the vaccines employed in the Philippines and Calcutta, respectively. Hence, the Philippine vaccines employed in the two field trials do not appear to be appreciably different based on comparisons from the Chiba Serum Institute and DBS data.

4. Passive protection studies in infant rabbits

Data from the Haffkine Institute on passive protection of intra-intestinally challenged infant rabbits which had received a total of 3.0 ml antiserum from adult rabbits hyperimmunized with the four Calcutta field trial vaccines, the ORL field trial vaccine and TAB vaccine, are shown in Table 9. When the 95 per cent. confidence intervals for the mean survival time between two groups of animals do not overlap, their mean survival time is taken as significantly different. The mean survival time of all the groups of antisera treated animals was compared with untreated control groups whose survival time was kept within definite narrow limits, irrespective of the challenge dose or type of vibrios (classic or El Tor). Animals receiving classical cholera antisera were challenged with classical vibrios, but those receiving the Philippine El Tor antiserum were challenged with El Tor strains. While few animals survived, all of the sera except TAB produced a significant increase in mean survival time between serum treated and untreated rabbits. Based on survival time, there is some suggestion that the WRAIR vaccine may be more protective against Ogawa challenge, and the ORL vaccine against Inaba challenge, than the other vaccines. None of these antisera, however, offered the type of absolute protection against death seen in earlier Haffkine Institute studies with live enteric immunizing antigens.

Efforts to demonstrate passive protection of orally challenged infant rabbits were also made at the DBS. Antiserum pools representing 14th and 56th day (after start of immunization) bleedings from rabbits hyperimmunized with the CRL, WRAIR and Haffkine vaccines were administered in a 1.0 ml/100 g of body-weight dose.

Table 10 shows the results of passive protection studies against Ogawa challenge. None of the antisera offered significant protection against death when compared with the control group. It is obvious that a much larger number of animals would be required to give statistically significant data based on survival with the low or negligible level of protection involved. Included in the table of comparison are results from protection studies conducted earlier using serum pools prepared from animals immunized with living vibrios. In this instance, there was an apparent increase in protective activity with pools representing sequential stages of the immune response. The 14- and 56-day bleedings from rabbits, immunized with the field trial vaccines are roughly comparable in regard to immunization schedule to pools 2 and 10, but no similar significant increase in protective power was observed.

An attempt was also made to demonstrate protective activity based on a possible extension of survival time in antiserum treated animals which eventually succumbed to cholera. The variations in survival time observed in both serum treated and control animals were rather great, hence no significant increase in survival time could be shown. Variations in survival time following intra-intestinal challenge appear to be less.

Results of a similar effort to show protection against Inaba challenge are shown in Table 11. Fewer survivors were obtained in serum-treated groups than with Ogawa-challenge, and no significant increases in survival times were observed.

5. Immunologic tests on sera from immunized animals

The results of agglutinating and vibriocidal antibody tests and antitoxin titrations (performed by Craig's skin test method - Nature (Lond.), 207, 614-616, 1965) on the serum pools employed in the DBS infant rabbit studies are given in Table 12. For serum pools from rabbits receiving field trial vaccines, agglutinating and vibriocidal titres were somewhat higher with 14- than with 56-day bleedings; and they are similar to titres of the sera against live vibrios (Pools 1, 2, 6 and 10). Hence, it is apparent that the protective activity of antiserum for the infant rabbit is not directly correlated with either agglutinating or vibriocidal antibody titre.

There is no evidence of a rise in antitoxin between 14- and 56-day serum pools from animals immunized with field trial vaccines and these sera are only slightly and probably insignificantly higher in antitoxin than pooled normal rabbit serum. By contrast there is a demonstrable increase in antitoxin levels with sequential pools, 2, 6, and 10, which correlates with increasing infant rabbit passive protective activity of the pools.

Serum pools employed in these DBS studies were also titrated at the University of Chicago for antitoxin content by the rabbit ilial loop method and the State University of New York by the skin test procedure, and the results are shown in Table 13. The "antitoxin units" in which the results are expressed are not directly comparable. As with the Division of Biologics Standards studies (Table 12), a difference of antitoxin level was found with pools 1 and 10 (anti-live vibrio) by both methods. With the skin test procedure no antitoxin was detected with the three field trial vaccine serum pools. Antitoxin was detected in these anti-vaccine sera by the ilial loop, but there is no evidence of an increase in titre between bleedings, and it is of interest to note that the antitoxin level in the sera from animals receiving Haffkine Vaccine was lowest. This comparison suggests that there may be some difference either in the lower threshold sensitivity levels of the two titration systems, or that possibly different toxic mechanisms are involved.

The Pasteur Institute has provided data on the agglutin and vibriocidin response of rabbits to a single 0.25 ml subcutaneous dose of each vaccine. Ten rabbits were immunized with each vaccine and were bled at intervals as shown in Table 14. For agglutinins there was no significant difference in the response to the different vaccines at the time intervals tested. However, with WRAIR and Philippine vaccines, a significant delay in appearance of maximum vibriocidal antibody titres was observed (cf. 14- and 28-day tests) as compared with Haffkine and Kasauli vaccine. However, the titres were comparable for all four vaccines at subsequent test intervals. Differences in Ogawa and Inaba titres were generally insignificant. The Pasteur Institute is also performing passive mouse protection tests on some of these sera, but results are incomplete.

DISCUSSION

Of the four field trial vaccines involved, it is apparent that the WRAIR vaccine was highest in bacterial content as judged from nitrogen and toxicity assays. Of course, this vaccine was intentionally prepared in this fashion, and this seems also reflected in its mouse protective activity, particularly in the data from DBS.

In view of the possible correlation of mouse toxicity and untoward reactions in man reported earlier (Focley & Pittman, *Lancet*, 1, 449-450, 1965), it is interesting that the WRAIR vaccine apparently did not produce any unusual incidence of reactions in the field. At the one-half single human dose level (0.5 ml) in the mouse, this vaccine approaches the toxicity of the CRL vaccine at this test level (i.e. 0.2 ml). At the 0.4 ml human dose, the CRL vaccine was just below the level of non-acceptability, since 0.5 ml doses and above produced frequent reactions. By comparison, the WRAIR vaccine at the 1.0 ml human dose level should be near the threshold of the maximum tolerated dose.

As was the case with the 1964 Calcutta field trial, a meaningful correlation of the results of laboratory assays with prophylactic efficacy in man will not be possible due to the low statistical significance of the field trial data caused by a low incidence of cholera among vaccines. Preliminary data for the first phase of the field trial show the following incidence rates (in cases per 10 000): Kasauli 10.2; Haffkine 13.6; TAB 17.0. On this basis, the Kasauli vaccine would appear about 40 per cent. effective and the Haffkine about 20 per cent. However, statistically speaking, protection from neither cholera vaccine is significantly different from TAB.

On the basis of mouse protection tests in the DBS and the "Human" Institute for Serobacteriologic Research, the Kasauli vaccine appears of somewhat higher potency than the Haffkine vaccine, but a valid comparison with field trial data is impossible.

None of the other studies (infant rabbit or immunologic tests) reveal any suggestion of differences between these two vaccines.

In the second phase of the field trial, preliminary data show the following incidence rates (cases per 10 000): WRAIR 7.91; Philippines 9.40; TAB 8.66. While the incidence of cholera is even lower than in the first phase, there is hardly even a suggestion of protection from these two vaccines.

Based on these second phase field trial results, one can only note that the WRAIR vaccine was more potent in the mouse assay than the similar WRAIR vaccine of lower bacterial content which gave at least some protection in the 1964 Calcutta field trial (see WHO/BS/774.65). Further, it is worth noting that the WRAIR vaccine was at least equal in potency, on a comparable human dose basis, to the vaccine employed in the CRL field trial. Also, the Philippine vaccine seems to be similar in mouse potency to the vaccine used in the 1964 Philippine field trial. The WRAIR and Philippine vaccines used in Calcutta in 1965 seem to be significantly different in mouse potency.

The antibody response data from the Pasteur Institute (see Table 14) should be noted in relation to the apparent lack of protection of the second phase vaccines. Both the WRAIR and Philippine vaccines gave a significantly delayed vibriocidal antibody response as compared with the Haffking and Kasauli vaccines. The significance of this observation is unknown, but perhaps further study is indicated.

On the basis of the present data it does not seem possible to conclude at this time whether any of the laboratory studies reported can reliably reflect the prophylactic efficacy of vaccines for man. This question can be answered only by future laboratory studies of vaccines which are employed in field trials giving results of adequate statistical significance.

LIST OF PARTICIPATING LABORATORIES
PROVIDING DATA USED IN THIS REPORT

1. Dr J. C. Feeley and Dr M. Pittman, Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland
2. Dr J. P. Craig, State University of New York Medical Centre, Brooklyn, New York
3. Dr W. Burrows, University of Chicago, Chicago, Illinois
4. Dr H. Ogonuki, Chiba Serum Institute, Ichikawa City, Japan
5. Dr A. K. Thomas, Central Research Institute, Kasauli, India
6. Dr N. K. Dutta, Haffkine Institute, Bombay, India
7. Dr I. Joo, "Human" Institute for Serobacteriological Production and Research, Budapest, Hungary
8. Dr J. Gallut, Pasteur Institute, Paris, France

TABLE 1. NITROGEN CONTENT OF FIELD TRIAL VACCINES

Vaccine	Total nitrogen, mg/ml of vaccine		
	Whole vaccine	After dialysis	Acetone precipitable*
Kasauli	0.33 (0.41)**	0.080	0.045
Haffkine	1.27 (1.25)	0.050	0.025
Philippine El Tor	0.12	0.050	0.030
WRAIR	0.64 (0.70)	0.250	0.250
CRL***	1.32	0.099	0.10

* Nitrogen content of material precipitated from 1 ml of vaccine by 1.5 ml of acetone.

** Values in parentheses are data supplied by "Human" Institute for Serobacteriological Production and Research, Budapest. Other data are from Division of Biologics Standards.

*** Vaccine used in Pakistan - SEATO Cholera Research Laboratory (CRL) Field Trial.

TABLE 2. FREEDOM-FROM-TOXICITY TESTS IN MICE ON FIELD TRIAL VACCINES*

Vaccine	Dose ml	Average weight change on day				
		1	2	3	7	S/10**
Kasauli	0.5***	-0.8	+0.5	+1.2	+5.5	10
	0.25	-0.7	+0.9	+2.1	+6.8	10
Haffkine	0.5**	-1.0	+0.6	+1.8	+5.9	10
	0.25	-0.7	+0.7	+1.8	+6.1	10
Philippine El Tor	0.5**	-0.5	+1.0	+1.8	+5.8	10
	0.25	-0.3	+0.9	+1.9	+5.6	10
WRAIR	0.5**	-1.3	-0.3	+0.8	+5.2	10
	0.25	-1.3	+0.3	+1.6	+5.8	10
CRL	0.2**	-1.4	-0.3	+0.1	+4.7	10
Saline	0.5	+1.0	+2.0	+3.0	+6.8	10

* Data from Division of Biologics Standards.

** S/10 = Survivors/10 mice injected.

*** = 1/2 single human dose.

TABLE 3. DIVISION OF BIOLOGICS STANDARDS MOUSE PROTECTION TEST DATA
1965 CALCUTTA FIELD TRIAL VACCINES
(Data based on 4 replicate tests)

Challenge strain	Vaccine	ED50, ml			Slope		
		Log	± log S.D.	ml × 10 ⁻⁴	S.D., %*	**c	± S.D.c
Ogawa NIH 41	Kasauli	-5.917	0.0891	0.826	82-123	1.04	0.170
	Haffkine	-4.070	0.0900	1.17	81-123	0.944	0.157
	Philippine	-4.179	0.0953	1.51	80-125	0.852	0.150
	WRAIR	-5.526	0.0949	0.336	80-124	0.852	0.150
	CRL	-5.345	0.0847	0.221	82-122	1.04	0.165
	U.S. Ogawa Ref.	-4.178	0.0717	1.51	85-118	1.23	0.178
Inaba NIH 35A3	Kasauli	-5.928	0.115	0.847	77-130	0.778	0.152
	Haffkine	-4.080	0.115	1.20	77-130	0.720	0.145
	Philippine	-4.193	0.0979	1.56	80-125	0.821	0.148
	WRAIR	-5.643	0.12	0.440	76-132	1.648	0.140
	CRL	-5.301	0.0738	0.200	84-119	1.26	0.187
	U.S. Inaba Ref.	-4.687	0.0869	4.86	82-122	0.952	0.156

* Limits of 1 S.D., expressed in per cent.

**c = slope coefficient of dose response.

TABLE 4. RELATIVE POTENCY OF 1965 CALCUTTA VACCINES
BY THE MOUSE PROTECTION TEST

(Data from Division of Biologics Standards)

Challenge strain	Vaccine	Relative potency		
		Per millilitre		Per 0.4 ml human dose*
		U.S. Reference = 1.00	CRL Vaccine = 1.00	CRL Vaccine = 1.00
Ogawa NIH 41	Kasauli	1.83** (1.08-3.09)***	0.268*** (0.152-0.427)	0.670 (0.381-1.18)
	Haffkine	1.29 (0.760-2.19)	0.189*** (0.107-0.335)	0.472*** (0.267-0.835)
	Philippines	1.00 (0.578-1.73)	0.146*** (0.0812-0.263)	0.365*** (0.203-0.657)
	WRAIR	4.49*** (2.60-7.77)	0.658 (0.366-1.18)	1.64 (0.913-2.95)
	CRL	6.83*** (4.10-11.4)	1.00 -	1.00 -
Inaba NIH 35A3	Kasauli	5.74*** (2.96-11.1)	0.236*** (0.126-0.444)	0.590 (0.314-1.11)
	Haffkine	4.05*** (2.09-7.86)	0.167*** (0.0890-0.314)	0.418*** (0.223-0.786)
	Philippines	3.12*** (1.71-5.71)	0.120*** (0.0728-0.225)	0.320*** (0.182-0.563)
	WRAIR	11.0*** (5.54-21.9)	0.455*** (0.237-0.874)	1.14 (0.593-2.19)
	CRL	24.3*** (14.4-41.1)	1.00 -	1.00 -

* CRL vaccine was employed in field trial mainly in 0.4 ml human dose; others were given in 1.0 ml dose.

** Significantly different from reference vaccine at 5% level or less.

*** Numerals in parentheses are 95% confidence limits.

TABLE 5. SUMMARY OF RESULTS OF MOUSE PROTECTION TESTS PERFORMED BY
"HUMAN" INSTITUTE FOR SEROBACTERIOLOGICAL PRODUCTION AND
RESEARCH

Challenge Strain	Vaccine	ED ₅₀ × 10 ⁻⁴ *	Relative Potency Haffkine = 1.0
Ogawa NIH 41	WRAIR	3.22	2.6
	Kasauli	4.10	2.0
	Haffkine	8.30	1.0
Inaba NIH 35A3	WRAIR	2.28	2.9
	Kasauli	2.72	2.5
	Haffkine	6.65	1.0

* Based on geometric mean value from four replicate tests performed.

TABLE 6. COMPARISON OF MOUSE PROTECTION DATA FROM DIVISION OF BIOLOGICS STANDARDS AND "HUMAN" INSTITUTE OF SERO-BACTERIOLOGICAL PROTECTION AND RESEARCH

Challenge Strain	Vaccine	Relative Potency "DES"	(Haffkine = 1.0) "Human"
Ogawa NIH 41	WRAIR	3.5	2.6
	Kasauli	1.4	2.0
	Philippine	0.78	NT*
	Haffkine	1.0	1.0
Inaba 35A3	WRAIR	2.7	2.9
	Kasauli	1.4	2.5
	Philippine	0.77	NT
	Haffkine	1.0	1.0

* NT = Not tested.

TABLE 7. COMPARISON OF WALTER REED AND KASAULI VACCINE BY THE MOUSE PROTECTION TEST*

Challenge Strain	Vaccine	ED ₅₀ ** mlx10 ⁻⁴	Rel. Potency (WRAIR = 1.0)
Ogawa NIH 41	Kasauli	0.413 (67-150)	0.81
	WRAIR	0.335 (62-162)	1.0
Inaba NIH 35A3	Kasauli	0.462 (46-151)**	7.9
	WRAIR	3.63 (58-171)	1.0

* Data from Central Research Institute, Kasauli.

** Data based on a single test of each serotype in which vaccines were tested concurrently.

TABLE 8. COMPARISON OF PHILIPPINE EL TOR VACCINE USED IN PHILIPPINE FIELD TRIAL (1964) AND CALCUTTA FIELD TRIAL (1965), BY MOUSE PROTECTION TEST ^a

Challenge Strain	Vaccine used in	ED ₅₀ ^b ml X10 ⁻⁴	Rel. Potency (Philippine = 1.0)
Ogawa El Tor 17	Calcutta	0.954 (73-138) ^c	0.47 ^d
	Philippines	0.448 (79.127)	1.0
Inaba El Tor V 86	Calcutta	2.67 (72-139)	1.4
	Philippines	3.81 (68-148)	1.0

^a Data from Chiba Serum Institute

^b Calculated from Combined Data based on two tests (Total 32 animals per dose).

^c () = range of 1 S.D. in per cent.

^d Difference from 1.0 is of borderline significance at 5% level.

TABLE 9. HAFFKINE INSTITUTE STUDIES ON
EFFECTIVENESS OF ANTISERA AGAINST DIFFERENT VACCINES IN
PROTECTING INFANT RABBITS AGAINST INTESTINAL CHALLENGE
OF HOMOLOGOUS VIBRIOS

Serum Against Vaccine	Challenge Strain	95% Confidence Limits for Mean Survival Time (Hours)		Significance ^a (Treated vs. Untreated).
		Serum Treated	Untreated	
Haffkine	Inaba	39.8-43.2 (0/8) ^b	24.9-29.4 (0/7)	S
	Ogawa	36.6-42.8 (0/10)	24.5-26.9 (0/10)	S
Kasauli	Inaba	40.2-42.4 (0/9)	26.8-29.8 (0/7)	S
	Ogawa	38.8-42.2 (0/8)	25.1-28.0 (0/8)	S
WRAIR	Inaba	40.3-56.2 (0/9)	26.1-29.3 (0/8)	S
	Ogawa	52.4-89.8 (1/10)	24.5-27.5 (0/8)	S
Philippine (El Tor)	Inaba El Tor	34.4-44.8 (0/13)	26.2-28.2 (0/10)	S
	Ogawa El Tor	38.9-41.2 (0/10)	25.4-27.8 (0/10)	S
CRL	Inaba	56.3-90.0 (2/12)	28.1-29.6 (0/8)	S
	Ogawa	31.4-39.8 (0/10)	24.1-27.4 (0/8)	S
TAB	Inaba	25.2-28.0 (0/10)	24.8-27.4 (0/10)	NS
	Ogawa	26.1-32.7 (0/10)	24.6-27.4 (0/10)	NS
	Inaba El Tor	23.9-29.7 (0/5)	23.3-29.1 (0/5)	NS
	Ogawa El Tor	22.9-28.3 (0/5)	25.3-28.1 (0/5)	NS

^a S = Significant. NS = Not Significant

^b () = Survivors/No. Challenged

TABLE 10. DIVISION OF BIOLOGICS STANDARDS STUDIES ON
PASSIVE PROTECTION OF INFANT RABBITS AGAINST OGAWA VC12 CHALLENGE

Pooled serum against	Pool No.	Bleeding day	S/n ^a	Survival time (hours) of dying animals		
				Medium	Range	Mean
CRL Vaccine	14A 14B	14 56	4/9 (0.18) ^b 0/8 (0.32)	23 26	22-47 20-46	29 (7-50) ^c 31 (10-52)
WRAIR Vaccine	15A 15B	14 56	0/9 (0.29) 3/9 (0.30)	24 22	23-70 19-41	37 (0-77) 27 (5-48)
Haffkine Vaccine	16A 16B	14 56	1/9 (0.43) 3/9 (0.30)	42 25	21-70 23-29	42 (3-81) 26 (21-31)
(Controls)	-	-	2/11 -	29	21-45	29 (13-45)
Live Ogawa VC12 ^d	1 2 ^e 6 ^e 10 ^e	12 14 27 64	0/12 (0.68) 4/20 (0.10) 11/20 (<10 ⁻⁵) 19/21 (<10 ⁻⁸)	- - - -	- - - -	- - - -
(Controls)	-	-	1/25	-	-	-

^a S/n = Survivors/number challenged:

^b () = Probability of significant difference from controls.

^c () = 95% confidence limits of mean.

^d - Data from passive protection studies reported by Feeley (1965).

^e - Sequential bleedings from same rabbits.

TABLE 11. DIVISION OF BIOLOGICS STANDARDS STUDIES ON THE
PASSIVE PROTECTION OF INFANT RABBITS AGAINST INABA VC13 CHALLENGE

Pooled serum against	Pool No.	Bleeding day	S/n ^a	Survival time (hours) of dying animals		
				Medium	Range	Mean
CRL Vaccine	14A	14	1/9 (0.47) ^b	29	17-35	28 (15-40) ^c
	14B	56	2/9 (0.34)	25	23-30	26 (20-31)
WRATR Vaccine	15A	14	0/9 (0.40)	29	17-35	27 (15-39)
	15B	56	0/9 (0.40)	29	23-35	29 (18-39)
Haffkine Vaccine	16A	14	0/9 (0.40)	25	21-30	26 (20-32)
	16B	56	1/9 (0.47)	27	25-70	32 (2-63)
Control	-	-	2/16	25	18-32	26 (18-34)

^a S/n = Survivors/number challenged.

^b () = Probability of significant difference from controls.

^c () = 95% confidence limits of mean.

TABLE 12. DIVISION OF BIOLOGICS STANDARDS STUDIES ON
AGGLUTINATING, VIBRIOCIDAL AND SKIN TOXIN NEUTRALIZING TITRES OF SERUM POOLS

Serum pool against	Pool No.	Bleeding day	Titre (log 2)		
			Agglutinating	Vibriocidal	Antitoxin
CRL Vaccine	14A	14	13	18	3
	14B	56	11	15	4
WRAIR Vaccine	15A	14	13	19	4
	15B	56	12	17	4
Haffkine Vaccine	16A	14	13	18	3
	16B	56	12	16	3
Live Ogawa VC12 ^b	1	12	15	19	3
	2 ^a	14	16	19	2
	6 ^a	27	13	17	6
	10 ^a	64	13	17	8
Normal rabbit	-	-	<4	<4	2

^a Used in passive protection studies reported by Feeley (1965).

^b Sequential bleedings from same rabbits during immunization.

TABLE 13. UNIVERSITY OF CHICAGO AND STATE UNIVERSITY OF NEW YORK STUDIES ON
ANTITOXIN CONTENT OF RABBIT ANTISERA - SUPPLIED BY DIVISION OF
BIOLOGICS STANDARDS

Antigen	Serum Pool No.	Antitoxin Units/ml	
		Skin Test ^a	Ileal loop ^b
Live	1	2 (approx.)	95
Ogawa	10	114	312
CRL	14A	<0.3	121
Vaccine	14B	<0.3	138
WRAIR	15A	<0.3	294
Vaccine	15B	<0.3	138
Haffkine	16A	<0.3	37
Vaccine	16B	<0.3	29

^a - Data from Dr J. P. Craig, State University of New York (Based on two Determinations).

^b - Data from Dr W. Burrows, University of Chicago (Preliminary Results).

TABLE 14. PASTEUR INSTITUTE DATA ON AGGLUTININ AND VIBRIOCIDIN RESPONSE OF RABBITS TO A SINGLE DOSE OF VACCINE

Vaccine	Test antigen	Mean Titres by Day after Immunization													
		Agglutinins							Vibriocidins						
		0	14	28	42	98	180	0	14	28	42	98	180		
WRAIR	Inaba	0	390	430	400	183	6	4	184	480	30 000	6 900	480		
	Ogawa	0	530	410	277	116	6	9	210	458	140 000	6 900	520		
Philippine	Inaba	0	100	220	340	40	0	10	66	498	7 300	1 720	534		
	Ogawa	0	150	300	275	55	0	20	65	520	25 300	2 710	520		
Haffkine	Inaba	0	344	255	175	57	NC ^b	6	29 000	5 000	17 888	4 600	NC		
	Ogawa	0	227	200	143	78	NC	2	39 000	26 000	30 250	3 443	NC		
Kasauli	Inaba	0	595	530	265	115	NC	12	235 000	37 000	28 000	3 000	NC		
	Ogawa	0	440	475	215	127	NC	40	145 000	27 100	27 200	2 610	NC		

^a - 0.25 cc of Vaccine Subcutaneously; 10 rabbits for each vaccine.

^b - NC = Not complete.