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Live Oral Cholera Vaccine:

Report of a Trial on Human Volunteer Subjects

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Earlier laboratory studies on the possibility of basing a live oral cholera vaccine on a naturally avirulent strain of El Tor vibrio have shown that the strain is capable of multiplication in the gastrointestinal tract of rabbits resulting in the production of antibacterial and antitoxic immunity. In order to examine the safety and immunogenic value for human use of this proposed vaccine, a systematic trial has been carried out on 25 human volunteers. Following oral administration of the vaccine after neutralization of gastric acidity, the volunteers developed statistically significant increases in their vibriocidal antibody level. Copro-antibodies could also be demonstrated in stool samples within a week of vaccination Serum and copro-antibody levels persisted unchanged during the periods of observation of 6 and 3 months, respectively. None of the volunteers suffered any ill effect during the course of the trial. It has been concluded that the proposed vaccine is safe for human use and likely to protect against infection, although its protective value has still to be confirmed in a field trial.

The possibility of developing a live oral cholera vaccine with an avirulent culture of an El Tor vibrio strain isolated from a water source in Calcutta has been studied in the Indian Institute of Experimental Medicine. Mukerjee (1963) reported detailed results of laboratory studies on the antigenic composition, pathogenicity and immunogenic value of the apparently apathogenic El Tor strain in comparison with the well-known choleragenic vibrios belonging to *Vibrio cholerae* and *V. eltor* types. Further studies have shown that the vaccine strain is capable of multiplication in the gastrointestinal tract of rabbits and gives rise to antibacterial as well as to antitoxic immunity (Bhattacharya & Mukerjee, 1968; Bhattacharya, Narayanaswami & Mukerjee, 1968). The safety and immunogenic value of the vaccine remained to be verified in human trials as the earlier trial on 10 human volunteers in Calcutta had proved inconclusive (Mukerjee, 1965). A fresh trial was therefore undertaken to examine the safety and immunogenicity of the proposed vaccine in humans.

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MATERIALS AND METHODS

Vibrio strains

An apathogenic strain of El Tor vibrio No. EW-6 isolated from a water source in Calcutta in 1958 was used as the vaccine strain. This strain has been extensively used in earlier studies in this laboratory.

V. cholerae strain No. 569B obtained from the Haffkine Institute, Bombay, India, was used as positive control in pathogenicity tests in animals.

Tests on rabbits

Adult rabbits weighing more than 1250 g and infant rabbits 8–10 days old were used for pathogenicity tests (De & Chatterjee, 1953; Dutta & Habbu, 1955) to confirm the stability of the avirulent character of the vaccine strain.

Human volunteers

The trial was carried out on 35 human volunteers from members of the staff of the Indian Institute of Experimental Medicine; most of them had not had occasion to handle cholera cultures in the laboratory. Volunteers were selected at random from both sexes and all age-groups from 22 to 58 years. Altogether, 12 volunteers were never vaccinated, but 1 volunteer in each of the years 1950, 1951, 1964, 1965, 4 in each

of the years 1960, 1962, 1963, and 7 in 1966 were vaccinated.

All the volunteers were healthy at the time the vaccine was administered. Stool samples were collected from each person and microscopical and bacteriological examinations were carried out. A few of the volunteers were found to have *Entamoeba histolytica* cysts in their stools and appropriate treatment was given. None of the stool samples showed any vibrios on culture and they were also free from usual enteropathogens like shigellae and salmonellae. Blood samples were also collected from all volunteers and serum was separated and stored under refrigeration at 4°C.

Preparation of vaccine

The vaccine strain EW-6 was made streptomycin-resistant (using 1000 μg of streptomycin per ml) for convenience in distinguishing it from other El Tor strains present in the area. Roux flasks containing papain-agar medium were each inoculated with 3 ml of a 3-h broth culture. After overnight incubation at 37°C the flasks were washed with 10 ml of sterile phosphate-buffered saline (pH 7.2) containing 0.1% peptone (Sanyal & Mukerjee, 1968) as a stabilizer and the washings were stored under refrigeration at 4°C. Vibrios were found to retain their viability for several days in the saline solution. Vaccine was usually prepared 1–2 days before the administration of the first dose and stored for up to 10 days. At the time of preparation the volume was adjusted to give viable counts of 2×10^9, 8×10^9 or 4×10^{10} vibrios per ml as required. The viable count of the preparation was determined every alternate day during storage and found to be unchanged.

After the preparation of each lot of vaccine and a determination of viable count, a control animal passage was carried out to test the stability of its avirulent character. The entire process of strain maintenance and vaccine preparation was carried out in a sterile room separated from the rest of the laboratory in order to avoid contamination.

One volunteer (No. 9) of the second group excreted vibrio in his faeces after the first dose of vaccine. Vaccine was prepared with that human-passaged strain and after receiving a dose of the new vaccine another volunteer (No. 13) also excreted vibrios. Subsequent lots of vaccine were prepared with this twice-human-passaged strain. The intention in using the human-passaged strain was to examine whether the vaccine strain after human passage could acquire enhanced virulence or increased capacity for colonization in the human intestine.

Mode of administration

Each dose of vaccine was administered on an empty stomach and no food was allowed to the volunteers within 2 hours of an administration of vaccine. The resting acidity of the stomach which could have affected viability of the vaccine was neutralized by administration of 2 teaspoonfuls (i.e., about 8 ml) of aluminium hydroxide gel 15 min prior to administration of the vaccine. In order to maintain the neutral reaction in the stomach a further dose of 1 teaspoonful (about 4 ml) of aluminium hydroxide gel mixed with 2 fluid ounces (about 62 ml) of water was given 15 minutes after the dose of vaccine. Two doses of vaccine at an interval of 7 days were given to each volunteer. The first dose consisted of 8×10^9 viable vibrios and the second dose 4×10^{10} viable vibrios. However, the first batch of 4 volunteers had 3 doses, 2×10^9, 8×10^9 and 4×10^{10} viable cells, respectively, at intervals of 7 days. Thus the vaccine was administered to 25 volunteers numbered serially in 4 batches of 4, 6, 7 and 8 volunteers in the months of March and April 1967.

Bacteriological investigations

Stool samples. Stool samples were collected from each volunteer for 3 consecutive days after each dose of vaccine. Sterile containers were supplied to volunteers in cases where immediate transfer to the laboratory was possible; otherwise, sterile test-tubes containing tellurite-peptone water (Monsur, 1963) and a swab were supplied. For rapid identification of vibrios, dark-field microscopy was used (Benenson et al., 1964). About 4 g of stool were mixed with 10 ml of peptone water and incubated for 4 h at 37°C. A few loopfuls from the top level of fluid were transferred to another test-tube containing 5 ml of peptone water and then incubated for another 16 h. Plating was done at each stage, i.e., at 0 h, 4 h and 20 h. Plating was done from tellurite-peptone-water media after 1 h, 5 h and 20 h of incubation. The following culture media were used for plating:

(1) Papain agar (with and without 1000 μg/ml of streptomycin);

(2) Bile salt agar;

(3) MacConkey agar;

(4) Aronson's medium;

(5) TCBS medium.

Colonies that developed after overnight incubation were tested by direct examination and also by stereomicroscopic examination under oblique light (Finkelstein & Gomez, 1963). Suspect colonies were isolated and tested with cholera O serum and monospecific Ogawa O serum. Other bacteriological and biochemical confirmatory tests were also made as a routine measure. In some of the bacteriologically negative cases, stool samples obtained after a saline purgative had been administered were examined for the presence of viable vibrios according to the methods described above.

Examination of intestinal contents for the presence of vibrios. The fate of the live oral vaccine in the intestinal tract was followed by intubation with Ryle's tubes and polyvinyl tubing inserted through the nose. In all cases the tubes were introduced into an empty stomach and the descent of the tubes was followed by X-ray screening. The Ryle's tubes could go down only to the duodenum; fluid was aspirated after definite intervals of time and at given lengths of tube. The pH of the aspirated fluid was noted and cultures were made.

The polyvinyl tube had a mercury bag at one end and markings at different levels along its length. The mercury bag acted as a bolus so that it could be passed by peristaltic movements of the intestine and its progress could also be followed by X-ray screening. Usually it took 12–18 hours for the end of the tube to pass beyond the ileum; in those cases where it did so, the corresponding length of the tubes varied from 300 cm to 400 cm. Samples of fluid were aspirated at intervals of ½ h.

In a few cases, the tubes were introduced first and vaccine was administered when the tube had reached to the duodenum so that the progress of vaccine through the intestine could be followed. To provide sufficient nutrients for multiplication of vibrios in the intestine, the vaccine was administered in peptone-water medium in 2 cases and meat soup was also fed to these volunteers after the vaccine had been administered.

Immunological studies

Collection of blood. Blood samples were collected from the volunteers 7–10 days after administration of the last dose of vaccine. Follow-up samples were taken 12 weeks after the last dose and also after 6 months. Separated sera were stored under refrigeration at 4°C.

Vibriocidal titres of post-vaccination sera were determined alongside those of the corresponding prevaccination samples, following the technique of Finkelstein (1962).

Collection of stool samples for copro-antibody. About 8–9 days after administration of the final dose of vaccine stool samples were collected for titration of copro-antibody (Freter, 1965). In the morning the volunteers were given on an empty stomach a dose of 4 g of sodium bicarbonate followed after an interval of 15 minutes by a 16-g dose of Epsom salts ($MgSO_4.7H_2O$, U.S.P.). Ample drinking-water was available to the volunteers. The liquid stool samples were collected in sterile containers and immediately transferred to a refrigerator at 4°C or to an ice-box. A solution of 1N sodium hydroxide was used to adjust the pH of samples to a value of 8. The samples were then centrifuged at 20 000 rev/min in an ultracentrifuge for 30 min and the supernatant was filtered through coarse filter-paper and stored in a deep-freeze unit at −10°C. Usually, copro-antibody titrations were carried out on the second or third day.

Copro-antibody titration was done by the passive haemagglutination method (Mel et al., 1965). This technique was specially followed since titration of copro-antibody by direct agglutination or vibriocidal tests did not prove satisfactory. Thoroughly washed sheep erythrocytes were treated with an equal volume of a 0.005% solution of tannic acid in saline and incubated at 37°C for 10 min in a water-bath. The cell suspension was washed and resuspended in buffered saline of pH 7.2. Equal quantities of the tanned-cell suspension and the whole-cell lysate of a *V. cholerae* strain (1 ml of each) were mixed thoroughly by gently stirring them into 4 ml of buffered saline of pH 6.4 and the mixture was kept at room temperature (30°C–32°C) for 20 min. Control cells were prepared in the same way, adding 1 ml of saline instead of whole-cell lysate. Inactivated normal rabbit serum absorbed with an equal volume of washed sheep red blood cells and diluted 1 : 100 was used as diluent. The sensitized cell suspension was centrifuged for 5 min at 2500 rev/min and the supernatant decanted. The cells were then washed and resuspended in 1 ml of diluent and serial twofold dilutions of the inactivated absorbed antisera were prepared. Controls were made with the normal serum diluent and, for non-specific reactions, with the antiserum. Experiments in which any of these 2 checks showed positive results were discarded. The racks were kept at room temperature (30°C–32°C) and the results were noted at the end of 12 h.

RESULTS

Viable vibrios could be isolated from stool samples of only 4 of the volunteers—2 (No. 9 and No. 25) after the first dose and 2 (No. 1 and No. 13) after the second dose—between 24 h and 48 h after the administration of the vaccine. None of the other volunteers excreted viable vibrios in their stools, even after the administration of saline purgative.

Earlier attempts to obtain samples of intestinal contents with the help of a Ryle's tube were unsatisfactory since, in many cases, the tube did not pass

TABLE 1

STUDIES OF SPECIMENS OBTAINED AFTER INTUBATION OF VACCINATED VOLUNTEER SUBJECTS

Sample No. [a]	After which dose of vaccine	Hour of collection after vaccination	Length of tube (cm)	pH of samples	Results after cultivation		
					Direct	4-h peptone-water enrichment	20-h peptone-water enrichment
(1)	(2)	(4)	(5)	(5)	(6)	(7)	(8)
2	3rd			6	—	—	—
3	3rd			3	—	—	—
4	3rd			2	—	—	—
7	1st			3.5	—	—	—
8	1st			6	—	—	—
9	1st			7	+	+	+
9/1	2nd	30	140	5.4	—	—	—
9/2	2nd	30 ½	160	5.4	—	—	—
9/3	2nd	31	180	5.6	—	—	—
9/4	2nd	31 ½	200	5.8	—	—	—
10/1	2nd	30	130	6.2	—	—	—
10/2	2nd	31	145	6.2	—	—	—
10/3	2nd	31 ½	170	6.2	—	—	—
10/4	2nd	32	180	6.2	—	—	—
13/1	2nd	½	110	7.2	+	+	+
13/2	2nd	1	140	6.8	+	+	+
13/3	2nd	1 ½	170	6.5	+	+	+
13/4	2nd	2	200	6.5	—	—	—
13/5	2nd	4	250	6.5	—	*E. coli*	*E. coli*
14/1	2nd	1	100	6.8	+	+	+
14/2	2nd	1 ½	110	6.8	+	+	+
14/3	2nd	2	140	6.2	+	+	+
14/4	2nd	4 ½	160	6.2	—	—	—
14/5	2nd	5 ½	180	6.0	*E. coli*	*E. coli*	*E. coli*
22/1	1st	29	180	6.2	—	—	—
22/2	1st	30 ½	220	6.5	—	—	—
22/3	1st	32 ½	250	6.5	—	—	—
22/4	1st	36 ½	300	6.7	*E. coli*	*E. coli*	*E. coli*
22/5	1st	39	340	7	*E. coli*	*E. coli*	*E. coli*
23/1	2nd	1 ½	60	5.4	—	—	—
23/2	2nd	2 ½	80	3.8	—	—	—
23/3	2nd	3 ½	125	7.0	—	—	—
23/4	2nd	4 ½	165	5.8	—	—	—
23/5	2nd	6	235	6.2	—	—	—
23/6	2nd	8	250	6.4	—	+	+
23/7	2nd	10	280	6.4	—	+	+
23/8	2nd	12	290	6.8	—	+	+
23/9	2nd	14	300	6.8	—	+	+
23/10	2nd	16	320	7.0	—	+	+
23/11	2nd	18	340	7.0	—	+	+
25/1	2nd	1 ½	60	6.0	+	+	+
25/2	2nd	2 ½	60	5.2	+	+	+
25/3	2nd	5	70	5.1	+	+	+
25/4	2nd	7	120	6.4	—	—	—

[a] Specimens in samples 1–9 were collected by Ryle's tube and the remaining specimens by polyvinyl tube.

beyond the stomach and the pH of the samples varied from 2 to 6. A specimen which showed viable vibrios on culture could be obtained from only 1 of the 6 volunteers examined by intubation with a Ryle's tube. The results of intubation with polyvinyl tubing proved more satisfactory. Specimens could be obtained from much lower down in the intestinal tract, down to a distance of 340 cm from the nose. In some instances the end of the tubing obviously reached beyond the ileum, since aspiration drew out faecal material. The results of bacteriological examinations of the samples are given in Table 1. It may be seen from the results summarized in Table 2 that the vaccine strain remained viable in

TABLE 2

RE-ISOLATION OF THE VACCINE STRAIN FROM DIFFERENT PARTS OF THE GASTROINTESTINAL TRACT OF VOLUNTEERS AFTER INTUBATION WITH A POLYVINYL TUBE

Region of the gastrointestinal tract	No. of cases investigated [a]	No. of cases in which vibrios were isolated [a]
Stomach	4 (½, 1, 1 ½, 2 ½)	3 (½, 1, 2 ½)
Duodenum	4 (1, 1 ½, 2 ½, 2 ½)	3 (1, 1 ½, 2 ½)
Jejunum and ileum	7 (30, 31, 1 ½, 2, 29, 14, 5)	4 (1 ½, 2, 14, 5)
Beyond illeum	2 (39, 18)	1 (18)

[a] Numbers in parentheses indicate the time interval in hours between the administration of vaccine and the collection of specimens.

all parts of the gastrointestinal tract up to 18 h after administration of the vaccine. In only 2 of the 5 cases found to be bacteriologically positive on intubation could viable vibrios be isolated from the stool samples. This indicated that vibrios were killed after their passage into the colon. It is possible that the slightly acid reaction of the colon, as well as the action of commensal organisms, adversely affected the viability of the vaccine.

The results of vibriocidal antibody titration of the blood samples of the volunteers before and at different intervals after vaccination are given in Table 3. It may be seen from the table that there was significant rise in the titre of vibriocidal antibodies in the blood of all but 3 of the volunteers within 10 days after vaccination.

The results of copro-antibody titration are given in Table 5. It may be seen that 8–9 days after vaccination copro-antibodies appeared in the stools of all but 1 of the volunteers and were present for as long as 12 weeks with slight fall in titre in some of the cases.

TABLE 3

ANTIBODY TITRATION OF BLOOD SAMPLES OF VOLUNTEERS AFTER THE ADMINISTRATION OF LIVE ORAL VACCINE

Volunteer No.	Prevaccination vibriocidal titre	Post-vaccination vibriocidal titre after:		
		8-10 days	3 months	6 months
1	10^{-3}	10^{-4}	10^{-5}	10^{-3}
2	10^{-2}	10^{-4}	10^{-4}	10^{-4}
3	10^{-2}	10^{-4}	10^{-5}	10^{-5}
4	10^{-4}	10^{-6}	10^{-5}	10^{-5}
5	10^{-4}	10^{-5}	10^{-5}	10^{-4}
6	10^{-4}	10^{-5}	10^{-5}	10^{-5}
7	10^{-1}	10^{-3}	10^{-4}	10^{-4}
8	10^{-2}	10^{-3}	10^{-4}	10^{-5}
9	10^{-3}	10^{-4}	10^{-4}	10^{-5}
10	10^{-2}	10^{-5}	10^{-5}	10^{-5}
11	10^{-3}	10^{-5}	10^{-3}	10^{-4}
12	10^{-4}	10^{-5}	10^{-4}	10^{-4}
13	10^{-5}	10^{-5}	10^{-5}	10^{-3}
14	10^{-3}	10^{-4}	10^{-4}	10^{-4}
15	10^{-4}	10^{-5}	10^{-4}	10^{-3}
16	10^{-2}	10^{-3}	10^{-4}	10^{-3}
17	10^{-4}	10^{-5}	10^{-5}	10^{-3}
18	10^{-4}	10^{-4}	10^{-4}	10^{-4}
19	10^{-3}	10^{-4}	10^{-3}	10^{-5}
20	10^{-2}	10^{-3}	10^{-5}	10^{-2}
21	10^{-4}	10^{-5}	10^{-1}	10^{-4}
22	10^{-3}	10^{-3}	10^{-1}	10^{-3}
23	10^{-3}	10^{-4}	10^{-5}	10^{-3}
24	10^{-3}	10^{-4}	10^{-3}	10^{-4}
25	10^{-3}	10^{-4}	10^{-4}	10^{-5}

Comparative value of live and killed oral cholera vaccine

It has been found that after the administration of live oral vaccine only very few of the viable organisms could be isolated in the stool samples of the volunteers. It is possible that the immune reaction devel-

oped in the blood and intestine of volunteers was simulated by lytic products of vibrios. It was therefore necessary to compare the effects of killed cholera vaccine with those of the live vaccine. A study was undertaken to examine the immune reactions in volunteers after the administration of killed cultures of the same organism (EW-6) in the same manner as live vaccine. The results of killed oral cholera vaccine on the sera and copro-antibody titre as compared with their preimmunization level are given in Tables 6 and 7.

A statistical analysis of the immunizing value of the live and killed oral cholera vaccine has been made.

Live oral vaccine. The effect of vaccination on antibody titre of the blood is set out in Table 3 and summarized in Table 4. Briefly, 8–10 days after

TABLE 4

SUMMARY OF RESULTS OF ANTIBODY TITRATION OF BLOOD SAMPLES OF VOLUNTEERS AFTER THE ADMINISTRATION OF LIVE ORAL VACCINE [a]

Time after vaccination	No. of individuals with post-vaccination titre:			Total No. of individuals
	lower [b]	equal [b]	higher [b]	
8–10 days	0	3	22	25
3 months	2	7	16	25
6 months	3	8	14	25

[a] See Table 3.
[b] Than prevaccination titre.

vaccination the antibody titre had increased in 22 out of 25 volunteers vaccinated. The sign test shows that this increase is statistically significant at the 1% probability level. After 6 months, the excess of volunteer subjects with a higher post-vaccination titre over those with titre lower than before vaccination, was still significant (at the 5% probability level).

Prevaccination haemagglutination titres from stool samples are not reported. However, it is seen in Table 5 that, 8–9 days after vaccination, only 1 vaccinated individual out of 25 had a titre below 4 and more than half had a titre of 8 or more, while the highest prevaccination titre among the 10 volunteers vaccinated with killed vaccine was 2 (see Table 7). In so far as the 2 groups were comparable before vaccination, it can be concluded that a significant increase of the haemagglutination titre

TABLE 5

COPRO-ANTIBODY TITRATION OF STOOL SAMPLES OF VOLUNTEERS BY HAEMAGGLUTINATION TECHNIQUE AFTER THE ADMINISTRATION OF LIVE ORAL VACCINE

Volunteer No.	Post-vaccination haemagglutination titre after:	
	8–9 days	3 months
1	4	2
2	4	4
3	8	4
4	8	4
5	4	2
6	16	8
7	8	4
8	8	4
9	4	4
10	4	2
11	16	8
12	32	4
13	4	4
14	4	4
15	8	nil
16	8	4
17	8	8
18	4	2
19	4	4
20	8	4
21	2	nil
22	4	2
23	8	8
24	4	2
25	8	4

was produced by the live vaccine. Three months after vaccination, the distribution of changes in haemagglutination titre as compared with the corresponding 8–9 days post-vaccination titre was as follows:

Change in titre	*Number of individuals*
Increase	0
No change	7
Decrease	18
Total	25

TABLE 6

ANTIBODY TITRATION OF BLOOD SAMPLES OF VOLUNTEERS AFTER THE ADMINISTRATION OF KILLED ORAL VACCINE

Volunteer No.	Prevaccination vibriocidal antibody titre	Post-vaccination vibriocidal antibody titre (after 8–10 days)
26	10^{-6}	10^{-6}
27	10^{-1}	10^{-1}
28	10^{-5}	10^{-6}
29	10^{-5}	10^{-5}
30	10^{-5}	10^{-5}
31	10^{-6}	10^{-6}
32	10^{-5}	10^{-6}
33	10^{-5}	10^{-5}
34	10^{-5}	10^{-5}
35	10^{-5}	10^{-5}

In this interval of time, the titre decreased in a significant proportion of the vaccinated subjects. However, it is observed that the titre was still as high as 8 in 4 of the volunteers.

Killed oral vaccine. No significant post-vaccination increase of antibody titre of blood samples was observed among the 10 volunteers vaccinated with killed oral vaccine. The titre did not change in 7 individuals, it decreased in 1 and increased in 2 individuals (see Table 6). It is observed, however, that in this group of volunteers the *prevaccination* antibody titre was as high as (if not higher than) the *post-vaccination* titre of the group vaccinated with live vaccine (compare the second column of Table 6 with the third column of Table 3).

TABLE 7

COPRO-ANTIBODY TITRATION OF STOOL SAMPLES OF VOLUNTEERS BY HAEMAGGLUTINATION TECHNIQUE AFTER THE ADMINISTRATION OF KILLED ORAL VACCINE

Volunteer No.	Prevaccination haemagglutination titre	Post-vaccination haemagglutination titre (after 8–9 days)
26	2	2
27	nil	2
28	2	2
29	2	2
30	nil	nil
31	nil	nil
32	2	2
33	nil	4
34	nil	nil
35	2	2

The vaccination had practically no effect on the haemagglutination titre (see Table 7); the prevaccination and post-vaccination titres remained unchanged in 8 out of the 10 volunteers vaccinated. An increase was observed in 2 cases.

DISCUSSION

There was evidence that live vibrios were present throughout the intestinal tract although no untoward effect was produced in any of the 25 volunteers. This is reasonable proof that the proposed vaccine is safe for human use. In spite of 2 successive passages through the human intestine there was no change in the pathogenicity of the strain. There is therefore little likelihood of the emergence of a virulent strain after such vaccination.

After live oral cholera vaccination, the volunteers developed both humoral and copro-antibodies which indicates a status of immunity of the vaccinated individuals. After 1 course of vaccination, consisting of 2 weekly doses, the antibody titre in blood appears to last for at least 6 months. After this period, the titre of humoral antibodies appeared to fall to the preimmunization titre in 8 cases and in 3 cases the titre was lower than the preimmunization level. But in 14 (56%) of the volunteers, the serum antibody titre remained higher than it had been before immunization. This indicates that in the majority of the subjects humoral antibody lasted for more than 6 months. The value of humoral antibodies in protection against cholera has not been considered to be very high (Mukerjee, 1963). On the other hand, the local antibodies in the form of free faecal antibodies or antibodies present in the cells of the mucous membrane lining the intestinal tract are likely to play a more significant role in protection against the infecting organism. In the present study, most of the volunteers showed by the tests adopted the presence of copro-antibodies in low titre within a short time after live oral vaccination and they continued to show the presence of these copro-antibodies for up to 3 months—the period over which observation was continued.

The present findings indicate that the live oral cholera vaccine consisting of an avirulent El Tor culture is safe for human use and is likely to give rise to both local and systemic antibodies. But in order to confirm its protective value, it is necessary that a small-scale field trial of the efficacy of the vaccine should be carried out in a cholera-endemic area.

Oral administration of killed cultures did not result in the production of either serum or copro-antibodies. The presence of live organisms in the intestine therefore appears to be necessary to stimulate local as well as general antibody response.

In only 4 of the 25 volunteers could viable vibrios be isolated from the stool. This was apparently due to the inability of the vibrios to survive in the human colon, owing to the acid reaction or of presence of commensal organisms. Therefore, it is not possible to draw any definite conclusion about the multiplication of vaccine strain in the human intestinal tract. Data from a field trial may help to elucidate this point.

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RÉSUMÉ

VACCIN ANTICHOLÉRIQUE VIVANT ADMINISTRÉ PAR VOIE ORALE: COMPTE RENDU D'UN ESSAI SUR DES VOLONTAIRES

On a étudié chez 25 volontaires le degré d'innocuité et les propriétés immunogènes d'un vaccin anticholérique vivant obtenu par culture d'une souche de *Vibrio cholerae* biotype El Tor naturellement non pathogène.

Le vaccin a été donné à jeun après neutralisation préalable de l'acidité gastrique par administration d'un gel d'hydroxyde d'aluminium. La première dose vaccinale contenait 8×10^9 vibrions viables, la seconde, administrée 7 jours plus tard, 4×10^{10} vibrions. L'examen bactériologique d'échantillons du contenu intestinal prélevés par tubage au moyen de tubes en polyvinyle a montré la présence de vibrions viables dans toutes les portions du tractus intestinal (au moins jusqu'au niveau de l'iléon) pendant 18 heures après la prise du vaccin. Bien que les tentatives d'isolement du germe à partir des selles aient échoué chez la plupart des vaccinés, on a néanmoins obtenu des cultures positives chez 4 d'entre eux 24 à 48 heures après l'administration du vaccin. Ces isolats ont servi à préparer un nouveau lot de vaccin destiné aux derniers groupes de volontaires.

Dix jours après la vaccination, on a noté une hausse statistiquement significative des titres sériques d'anticorps vibriocides qui s'est maintenue pendant 6 mois. Des coproanticorps ont été décelés dans les selles 8-9 jours après la vaccination et ont persisté pendant 12 semaines. Aucun effet secondaire désagréable n'a été enregistré durant l'essai. Lorsque, aux fins de comparaison, des doses identiques d'un vaccin anticholérique tué ont été administrées par voie orale à 10 autres volontaires, il n'en est résulté ni élévation des titres sériques d'anticorps ni apparition de coproanticorps.

Les auteurs concluent que la préparation vaccinale vivante qu'ils ont utilisée est dépourvue de nocivité pour l'homme. En dépit de deux passages successifs dans le tractus intestinal, on n'a observé aucune augmentation du pouvoir pathogène de la souche. Le vaccin suscite la production d'anticorps sériques et de coproanticorps. Etant donné le rôle important que jouent ces derniers, il est probable que la vaccination confère une réelle protection contre l'infection. Cette protection devra cependant être confirmée au cours d'un essai pratique mené dans une région d'endémicité cholérique.

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