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Serological Studies in Cholera

3. Serum Toxin Neutralization—Rise in Titre in Response to Infection with *Vibrio cholerae*, and the Level in the "Normal" Population of East Pakistan *

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A method has been evaluated for the titration of antibodies to Vibrio cholerae, *based on the ability of sera containing such antibodies to neutralize the inflammatory effect of a factor from* V. cholerae *cultures on the skin of test animals.*

Ninefold or greater rises in toxin-neutralization titre were found in 73% of 111 bacteriologically confirmed cholera patients in an endemic area of East Pakistan, and in 2.5% of bacteriologically negative patients. This method compares well with the microtechniques developed for the titration of vibrio-agglutinating and vibriocidal antibodies to V. cholerae.

The toxin-neutralization method has the advantage that no titre rise is produced in response to vaccination with the whole-cell vaccine in current use in East Pakistan.

Relatively high toxin-neutralization titres were noted among children under 15 years of age without vibriocidal or agglutinating antibodies, and without a history of prior infection with V. cholerae.

Craig (1965a, 1965b, 1966) reported the presence of a factor in cholera stools and in filtrates of certain broth cultures of *Vibrio cholerae* which produced induration, erythema and increased capillary permeability at the site of intradermal injection in guinea-pigs or rabbits, reaching a peak at 18–24 hours. He reported that the responsible factor was neutralized by convalescent sera from confirmed cholera cases, but not by human sera with high agglutinin titres evoked by commercial cholera vaccine nor by rabbit sera with high titres evoked by the injection of living organisms grown on 1% tryptose slants. Feeley (personal communication, 1966) observed, however, that the sera of rabbits after prolonged immunization with agar-grown living vibrios did neutralize this toxic factor.

The procedure as outlined by Craig was incorporated into the serological procedures in this laboratory; this is a report of the results obtained when admission sera, convalescent sera, or both, of selected patients on a cholera ward were studied.

MATERIALS AND METHODS

Preparation of toxin

Filtrates were prepared by growing *V. cholerae*, Ogawa, CRL strain B1307, in 5% Difco Bacto-Peptone broth, pH 7.3, in Kolle flasks for 24 hours. The bulk of organisms was removed by centrifugation in the cold, and the supernatant fluid was sterilized by passage through a 0.22-μ membrane filter (Millipore Filter Company, Bedford, Mass., USA). The bacteria-free filtrates were divided into aliquots and stored at −20°C (with no loss of skin toxin activity over the duration of this study).

Preparation of animals

Albino guinea-pigs or rabbits were used. Rabbits were clipped, while guinea-pigs were clipped and depilated with a barium-sulfide paste. A grid was marked over the prepared back with indelible pencil or marker; approximately 90 sites were used on the adult rabbit, and about 25 on the guinea-pig. Animals were used only once.

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Titration of toxin

Each lot of toxin was titrated by injecting 0.1 ml of serial dilutions, in replicate, into the skin of the back of several guinea-pigs. Eighteen hours later, 0.12 ml of 5% Pontamine Sky Blue 6XB per 100 g body-weight was injected intravenously; after 1–1 ½ hours the injection sites were examined and the intensity and diameter of blueing recorded. The standard dose, i.e., the concentration in 0.1 ml required to produce a blue area with a diameter of 8 mm, was determined. For the routine test, the filtrate was used at twice this concentration of toxin, so that the standard dose was contained in 0.05 ml.

Sera

Serial bleedings from patients admitted to the ward associated with this laboratory were stored in the frozen state until used; daily rectal swabs were cultured for vibrios, and vibriocidal and agglutinating antibodies determined by a microtechnique as described earlier (Benenson, Saad & Paul, 1968; Benenson, Saad & Mosley, 1968).[1] Cases were selected at random for testing; however, all bacteriologically positive patients who failed to develop a rise in bacterial agglutinin, vibriocidal antibody, or both, were included preferentially for toxin-neutralization studies. Two or more sera of a patient were always run in the same test series and tested on the same animals.

Threefold serial dilutions of serum or plasma, starting at 1 : 10, were used in the routine test. The sera were not inactivated, since trial inactivations produced no effect on the titre or on the type of reaction elicited by positive and negative sera. In order to economize on animals, the sera were usually screened by testing 3 dilutions (1 : 10–1 : 90); sera were retitrated when the end-point was not reached. Usually the highest dilution tested was 1 : 2430.

Test procedure

0.2 ml of each serum dilution was mixed with an equal volume of toxin diluted to contain a standard dose in 0.05 ml. These mixtures, together with a control mixture of toxin diluted with saline (to ensure normal reactivity of the test animal), were incubated in a 37°C water-bath for 30 minutes. The samples were coded, and 0.1 ml injected intradermally in duplicate so that each mixture was tested both in the front and in the rear of the same animal.

After 18 hours, the injection sites were examined and induration recorded; 0.12 ml of 5% solution of Pontamine Sky Blue 6XB in 0.5 N saline per 100 g body-weight was then injected intravenously. After 1–1½ hours the intensity of blueing was recorded as + to ++++ and the diameter recorded in millimetres.

Interpretation of reactions

With a serum dilution which neutralized the toxin, the injection site was either not evident or merely represented by a point of blueing at the site of the needle insertion. The titre for each serum was considered to be the dilution at which the mean diameter of the resulting skin reactions was reduced in size to one-half or less that of the mean of the control sites. A ninefold titre rise was considered significant, a threefold rise equivocal and a change from negative to positive at the 1 : 10 dilution was considered to be within the experimental error. In calculations, all sera positive at 1 : 2430 or higher were assumed to have a titre of 2430, and all negative at 1 : 10 were assumed to have a titre of $3^1/_3$.[2]

RESULTS

Antitoxin response to infection

Paired sera (with the second serum drawn 6 or more days after the onset of symptoms) were examined from 111 patients from whom rectal swabs were positive for *V. cholerae* on 2 or more occasions, and from 40 patients from whom daily rectal swabs during the period of hospitalization were vibrio-negative. Nine of the 111 infections were caused by the Ogawa serotype and 102 by

[1] See the articles on p. 267 and p. 277 of this issue.

[2] Following the completion of this study, reference standard antitoxic serum prepared by Dr John P. Craig was kindly supplied for standardization of the toxin preparations used in this test. In terms of the Limit of Blueing units (LB_1) described by Craig (1966), the test dose of toxin used in this study contained 0.15 LB_1 unit of toxin. Thus it is possible to estimate the average number of antitoxin units per millilitre of serum that would be represented by the titres reported in this paper, as follows:

Titre	*Antitoxin units/ml*
<1 : 10	<1.5
1 : 10	2.7
1 : 30	8.1
1 : 90	24.3
1 : 270	72.9
1 : 810	218.7
1 : 2430	656.1

TABLE 1

RISE IN TOXIN-NEUTRALIZATION TITRES IN VIBRIO-POSITIVE AND VIBRIO-NEGATIVE DIARRHOEA PATIENTS [a]

V. cholerae swab	Age-group (years)	No. examined	Rise in titre					
			≥2 Dilutions (9×)		1 Dilution (3×) only		<1 Dilution (<10–10)	No rise
			No.	%	No.	%		
Positive	0–14	54	39	72	9	17	—	6
	≥15	57	42	74	12	21	2	1
	Total	111	81	73	21	19	2	7
Negative	0–14	13	—	—	1	8	—	12
	≥15	27	1	4	—	—	4	22
	Total	40	1	2.5	1	2.5	4	34

[a] Second serum of pair taken 6 or more days after onset of disease.

the Inaba serotype. Sera from patients with a single positive rectal swab were excluded from this analysis to eliminate any possible false positive cases.

A threefold or greater increase in toxin-neutralizing activity developed in 92% of the vibrio-positive cases; in 73% the increase was ninefold (2 dilutions) or more (Table 1). The proportion with a titre rise of ninefold or more was practically the same in those aged 15 years or over (74%) and those under 15 years of age (72%). Nine bacteriologically confirmed cholera patients developed no rise in toxin-neutralization titre, by the seventh (2 cases), eighth (3), ninth (1), tenth (2) and fourteenth (1) days; 6 of these were children of whom 2 were under 1 year of age (Table 2). Four of these 9 patients also failed to develop a rise in vibriocidal or agglutinating antibodies. Two had toxin-neutralization titres of 1 : 270 on admission, which could conceivably have obscured a specific titre rise. All these patients had severe clinical disease; one required intravenous fluid as late as the ninth day after the onset of symptoms.

There were 2 among the 40 vibrio-negative patients who showed a titre rise. One, with a ninefold rise in titre, also had a significant rise in vibriocidal titre against the Inaba antigen, while the other had been vaccinated 7 days earlier which very often indicates cholera in the local community. Although contact with a known cholera patient could not be established in either case, it cannot be excluded. These cases, which may thus well represent bacteriological failures, are taken here as false positives.

The toxin-neutralizing antibody appeared early. Retrospective analysis of bacteriologically confirmed cholera cases with toxin-neutralizing antibody rises indicated that the titre rise occurred in most cases by the end of the first week after the onset of disease. One patient showed a rise in titre from 30 to 270 between the second and fourth day after reported onset; on the fifth day, 1 of the 5 patients tested showed a rise in titre, but on the sixth day 9 out of 10 had had a significant rise. A titre rise was demonstrable in 90% or more of the sera taken on the seventh, eighth and ninth days after onset. Only 3 of the 9 bacteriologically positive patients who failed to show a rise in toxin-neutralizing antibodies were examined on the tenth day or later (Table 2).

Several serum samples were examined from 29 patients; in these the peak antibody level was found in the second to third week and there was a fall in titre of one-third which became apparent after the twenty-first day. Sera taken up to 74 days after onset had shown a fall in titre of 2 to 3 dilutions from the peak level; after this interval residual titres ranged from 10 to 270.

Toxin-neutralizing levels in the "normal" population

Analysis of the acute and convalescent titres of the non-cholera cases (Table 3) revealed a titre range

TABLE 2

DISCREPANT TOXIN-NEUTRALIZATION RESPONSES: BACTERIOLOGICALLY POSITIVE CASES WITH NO RISE IN TOXIN-NEUTRALIZATION TITRE, AND BACTERIOLOGICALLY NEGATIVE CASES WITH TITRE RISE

Age	Day of serum collection after onset of disease		Serological titre [a]			Antibiotic given	Vibrio isolations: serotype (and No. of days positive)
	Acute	Convalescent	Toxin-neutralization	Bacterial agglutination	Vibriocidal		
Bacteriology positive—no rise in toxin-neutralization							
months							
1½	3	10	10–10	10–10	10–10	Tetracycline	Inaba (2)
11	1	10	270–270	<10–<10	<10–<10	Tetracycline	Inaba (6)
years							
1	1	14	10–10	<10–<10	<10–<10	Chloramphenicol	Inaba (10)
3	1	8	270–270	<10–<10	10–40	Tetracycline	Inaba (2)
3	1	7	30–30	<10–20	<10–320	Tetracycline	Inaba (2)
4	2	8	10–10	<10–10	<10–<10	Tetracycline	Ogawa (2)
22	3	7	30–30	<10–40	<10–160	Chloramphenicol	Inaba (3)
45	1	8	<10–10	10–80	40–320	Tetracycline	Inaba (3)
45	1	9	<10–10	10–80	80–1280	None	Inaba (1)
Bacteriology negative—toxin-neutralization rise							
6	2	6	30–90	80–80	320–320	None [b]	Negative
45	1	9	<10–30	40–20	320–>1280 (Inaba only)	None [c]	Negative

[a] Acute-phase titre—convalescent titre.
[b] Cholera vaccine 7 days before first bleeding.
[c] No vaccine history.

TABLE 3

ACUTE AND CONVALESCENT TOXIN-NEUTRALIZATION TITRES IN NON-CHOLERA CASES [a]

Convalescent titre	Acute-phase titre					Total No. of cases
	<10	10	30	90	270	
270	—	—	—	—	1	1
90	—	—	1	1	1	3
30	1	—	6	1	—	8
10	4	7	3	—	—	14
<10	13	1	—	—	—	14
Total	18	8	10	2	2	40

[a] Both the acute-phase and the convalescent titres had geometric mean values of 11 with a 95% confidence interval of 7–16.

from less than 10 to 270 in this "normal" population, with no significant change in the convalescent samples. In a similar analysis of the titres of vibrio-positive cases, where the number of cases was larger, it became obvious that children and adults should be analysed separately (Table 4). The geometric mean of the acute-phase titres of the children (those under 15 years of age) was significantly higher than of those aged 15 years or over (Student's $t=3.92$; 109 degrees of freedom); both groups developed a rise in toxin-neutralizing antibodies in convalescence from cholera to levels which did not differ significantly from each other ($t=1.32$; 109 degrees of freedom).

To permit better assessment of the effect of age on baseline toxin-neutralizing levels, 51 additional admission sera were selected to fill out the age-groups with few cases. Cases were picked, so far as possible, which were vibrio-negative and gave no

TABLE 4

ACUTE AND CONVALESCENT TOXIN-NEUTRALIZATION TITRES IN BACTERIOLOGICALLY POSITIVE CHOLERA CASES

Convalescent titre	Acute-phase titre					Total No. of cases	Mean titre (95 % confidence interval)
	<10	10	30	90	270		
Age 0-14 years							
≥2 430	—	1	—	2	2	5	
810	1	1	8	2	1	13	
270	3	1	7	4	2	17	
90	2	6	4	—	—	12	234 (148–348)
80	3	—	1	—	—	4	
10	—	2	—	—	—	2	
<10	1	—	—	—	—	1	
Total	10	11	20	8	5	54	
Mean titre (95 % confidence interval)	23 (15–34)						
Age 15 years and over							
≥2 430	—	2	2	1	1	6	
810	2	5	2	—	—	9	
270	7	2	3	—	—	12	
90	6	2	6	—	—	14	161 (106–233)
30	7	6	1	—	—	14	
10	2	—	—	—	—	2	
<10	—	—	—	—	—	0	
Total	24	17	14	1	1	57	
Mean titre (95 % confidence interval)	8 (7–12)						

history of cholera immunization (of the 51 selected, 36 were bacteriologically negative and 15 were positive for *V. cholerae*; 6 gave a history of having cholera vaccination). Analysis of the total of 232 admission sera (Table 5) revealed no difference in geometric mean titres between those with a history of vaccination and those without, and no difference in admission toxin-neutralizing titres between the bacteriologically confirmed cholera patients and those who remained consistently vibrio-negative, or between those in the 0–4-year and the 5–14-year age-groups, but there was a marked difference in titres between those under 15 years and those aged 15 years or over ($t=6.4$; 230 degrees of freedom).

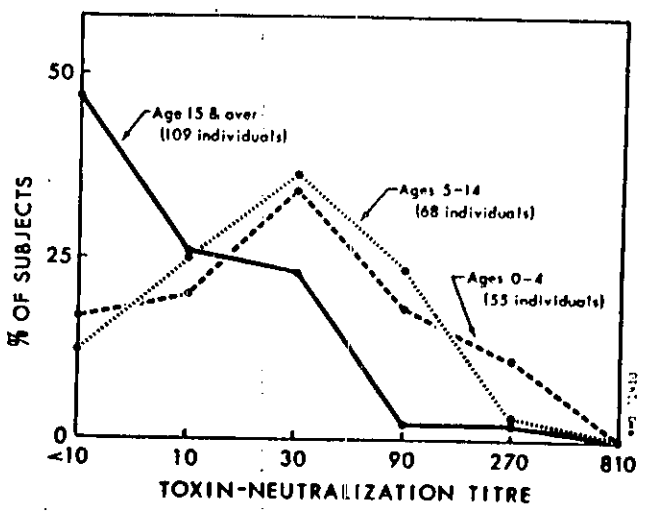
The percentage distribution curves for the titres in the various age-groups (see the accompanying figure) revealed no essential difference in pattern between the 0–4-year and 5–14-year age-groups. (The 5–14-year age-group contained 39 children aged 5–9 years, and 29 aged 10–14 years; these subgroups did not differ from each other). However, the pattern of toxin-neutralizing titres in the adult group was clearly different; almost 50% of the admission sera of those aged 15 years or over contained no toxin-neutralizing antibody detectable at a 1 : 10 dilution and only 5.5% had a titre of 90 or higher; while 25%–30% of the younger groups had titres of 90 or more, and 30 was the modal titre.

TABLE 5

MEAN TOXIN-NEUTRALIZATION TITRES IN ADMISSION SERA OF VACCINATED AND UNVACCINATED PATIENTS, BY AGE-GROUP AND PRESENCE OR ABSENCE OF VIBRIOS

Group	No. of cases	Toxin-neutralizing titre	
		Geometric mean	95% confidence limits
0–4 years	55	27	19 39
5–14 years	68	24	19–32
0–14 years	123	23	20–32
⩾15 years	109	9	7–11
0–14 years			
No vaccine	97	26	20–34
Vaccine	26	21	14–33
⩾15 years			
No vaccine	78	9	7–12
Vaccine	31	9	6–14
Cholera cases	91	15	12–19
Non-cholera cases	141	17	13–22
Total	232	15	13–19

FREQUENCY DISTRIBUTION OF ADMISSION TOXIN-NEUTRALIZATION TITRES, BY AGE



Relation of the antitoxic and vibriocidal titres

A comparison of the toxin-neutralization titre and the vibriocidal titre of the same admission sera revealed a poor correlation (Table 6). In contrast to the toxin-neutralizing titre, the geometric mean vibriocidal titre was significantly higher ($t=3.62$; 108 degrees of freedom) in the older age-groups than in the younger; this agrees with observations on another population group in East Pakistan (Mosley et al., 1968), and is consistent with prior exposure to the vibrio in this endemic area. During convalescence from proven cholera, both antibodies were usually present at a high level, but the correlation of the titres was poor. The mean [1] vibriocidal antibody level continued to be significantly higher in the older age-group ($t=3.76$; 109 degrees of freedom), while mean [1] toxin-neutralizing levels were still slightly higher (but not significantly so; $t=1.44$; 109 degrees of freedom) in the *younger* age-group.

Relative diagnostic efficiency of various serological methods

The relative efficiency of titrations for rise in toxin-neutralizing, vibriocidal and agglutinating antibody for the retrospective diagnosis of cholera was evaluated by comparing the respective results obtained with the patients whose sera were studied by each of the 3 methods. Among 111 patients bacteriologically positive for cholera (Table 7), 81 showed an antibody rise (9-fold) by the toxin-neutralization test, 98 by the vibriocidal test (4-fold) and 88 by the bacterial agglutinin test (4-fold rise). However, these results are not to be taken as the measure of the diagnostic efficiency of these methods in routine performance, since these cases were biased by the inclusion of all cholera patients who had had no titre rise in the bacterial agglutination and vibriocidal tests. Among the 40 vibrio-negative cases (Table 7), the toxin-neutralization test had the lowest incidence of false positives; it is to be noted that 2 of the false positive patients in the vibriocidal and bacterial tests were among 6 who had received vaccine within a week before admission. Thus, in this group of 151 cases, the toxin-neutralization test was rather less sensitive but more specific for detection of infection than either of the other 2 procedures. While threefold (one-step) titre rises cannot be considered significant (but equivocal, indicating the need for retitration over the indicated dilution range using serial twofold dilutions), if these

[1] For the calculation of mean values, vibriocidal titres of 1280 or higher were taken as 1280, toxin-neutralization titres of 2430 or higher were taken as 2430, and tests negative at 1:10 were considered to have a titre corresponding to one dilution lower (1:5 and 1:3½, respectively).

TABLE 6. RELATION OF TOXIN-NEUTRALIZATION AND VIBRIOCIDAL TITRES IN ACUTE-PHASE AND CONVALESCENT SERA OF CHOLERA CASES, BY AGE

TABLE 6. RELATION OF TOXIN-NEUTRALIZATION AND VIBRIOCIDAL TITRES IN ACUTE-PHASE AND CONVALESCENT SERA OF CHOLERA CASES, BY AGE

Vibriocidal titre	Acute-phase sera: Toxin-neutralizing titre <10	10	30	90	270	810	2430	Total No. of cases	Mean titre (95 % confidence interval)
Age 0–14 years									
1 280	—	—	—	—	—	—	—	—	
640	—	—	—	—	—	—	—	—	
320	—	—	—	—	1	—	—	1	
160	—	1	—	2	—	—	—	3	
80	2	—	3	—	—	—	—	5	11
40	—	—	1	—	—	—	—	1	(9–14)
20	1	1	3	1	—	—	—	6	
10	1	—	2	3	—	—	—	6	
<10	6	9	10	2	4	—	—	31	
Total	10	11	19	8	5	—	—	53	
Mean titre (95 % confidence interval)	23 (15–34)								
Age 15 years and over									
≥1 280	1	—	—	—	—	—	—	1	
640	1	—	—	—	—	—	—	1	
320	2	1	2	—	—	—	—	5	
160	3	1	2	—	1	—	—	7	
80	6	2	3	—	—	—	—	11	32
40	2	1	2	—	—	—	—	5	(21–49)
20	2	1	—	—	—	—	—	3	
10	—	5	—	—	—	—	—	5	
<10	7	6	5	1	—	—	—	19	
Total	24	17	14	1	1	—	—	57	
Mean titre (95 % confidence interval)	9 (7-12)								

Vibriocidal titre	Convalescent sera: Toxin-neutralizing titre <10	10	30	90	270	810	2430	Total No. of cases	Mean titre (95 % confidence interval)
Age 0-14 years									
≥1 280	—	—	2	3	3	7	—	15	
640	—	—	—	1	2	1	2	6	
320	—	—	2	4	3	1	1	11	
160	—	—	—	4	3	2	1	10	
80	—	—	—	—	2	—	—	2	242
40	—	—	—	—	1	2	1	4	(155–378)
20	—	—	—	—	1	—	—	1	
10	—	—	—	—	2	—	—	2	
<10	1	2	—	—	—	—	—	3	
Total	1	2	4	12	17	13	5	54	
Mean titre (95 % confidence interval)	224 (148–333)								
Age 15 years and over									
≥1 280	—	1	6	8	4	5	2	26	
640	—	1	4	1	1	2	2	11	
320	—	—	1	4	3	1	1	10	
160	—	—	2	—	—	1	1	4	
80	—	—	—	2	1	—	—	3	534
40	—	—	1	—	1	—	—	2	(398–720)
20	—	—	—	—	—	—	—	—	
10	—	—	1	—	—	—	—	1	
<10	—	—	—	—	—	—	—	—	
Total	—	2	15	15	10	9	6	57	
Mean titre (95 % confidence interval)	151 (102-224)								

TABLE 7

COMPARISON OF RISE IN TOXIN-NEUTRALIZATION, VIBRIOCIDAL AND BACTERIAL AGGLUTINATING TITRES FOR RETROSPECTIVE DIAGNOSIS OF CHOLERA

Serological results			No. of cases	
Toxin neutralization (9 × rise)	Vibriocidal (4 × rise)	Bacterial agglutinin (4 × rise)	Vibrio-positive	Vibrio-negative
Positive	Positive	Positive	69	0
Positive	Positive	Negative	10	1
Positive	Negative	Positive	0	0
Positive	Negative	Negative	2	0
Negative	Positive	Positive	17	3
Negative	Positive	Negative	2	1
Negative	Negative	Positive	2	0
Negative	Negative	Negative	9	35
Total			111	40
Percentage of cases positive by:				
Toxin neutralization			73	2.5
Vibriocidal activity (microtechnique)			88	12.5
Bacterial agglutination (microtechnique)			79	7.5

are taken into consideration the toxin-neutralization test suggested infection in 21 additional vibrio-positive cases, giving a total of 92%; on this assumption, therefore, the toxin-neutralization test suggested infection more frequently than either of the other 2 procedures; in only one vibrio-negative case was there a threefold tube rise, giving a false positive rate of 5%, which is still lower than for the other methods.

DISCUSSION

This study has indicated that the toxin-neutralization test can be incorporated into the diagnostic armoury of a routine laboratory. This animal assay, however, is more cumbersome than standard *in vitro* serological methods and the number of sera that can be processed is limited by the availability of appropriate test animals. The diagnostic value of the procedure lies primarily in its ability to discriminate between the antibody rises due to infection and those due to parenteral immunization with the ordinary currently available whole-cell or derivative vaccines which are free of cholera skin toxin; if and when cholera-vaccine production methods are changed so that an antitoxic response is elicited, this test will offer no more than the technically simpler vibriocidal test.

The presence of relatively high toxin-neutralization titres in children, in the absence of vibriocidal (and agglutinating) antibody, raises the question whether the ability of a serum to neutralize the toxic factor in a cholera culture filtrate is due to a specific antibody. If it were, one would expect the age distribution to be the reverse of that found, with the titre higher in the older age-group, which has more experience of infection, and not in the younger age-group. The relatively high toxin-neutralization titre persists with essentially no change up to the age of 15 years; but both the age incidence of clinical cholera and the levels of the vibriocidal and agglutinating antibodies suggests that the indigenous population in the Dacca area has attained immunity to cholera by age 10 years. It is evident that more investigations must be conducted to clarify these paradoxic findings.

Infection with the cholera vibrio evokes an increase in the toxin-neutralizing activity of the serum, and, since the action of the toxin on the skin may be analogous to the phenomena occurring

in the mucosa of the intestine, it is an attractive hypothesis that the symptoms of the disease abate when toxin-neutralizing antibodies appear (Craig, 1966). In this series, there were 9 cholera patients whose symptoms were brought under control in the absence of a rise in toxin-neutralizing antibodies demonstrable at the level of sensitivity of the test as performed; contrariwise, 13% had a toxin-neutralization titre of 1 : 90 or higher during the acute phase of the disease. In cholera cases not treated with antibiotic, the time at which antibody appears in the circulating blood does correlate very well with the time when symptoms abate (Greenough et al., 1964).

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RÉSUMÉ

Craig a signalé la présence dans les selles de cholériques et les filtrats de cultures de *Vibrio cholerae* d'une toxine qui, injectée dans le derme du lapin ou du cobaye, provoque localement une augmentation de la perméabilité des capillaires, de l'érythème et de l'induration. Ce facteur est neutralisé par les anticorps contenus dans le sérum des convalescents de choléra.

Les auteurs ont mis au point et expérimenté une méthode de recherche des anticorps neutralisant la toxine. L'examen des sérums couplés prélevés chez 111 malades atteints de choléra confirmé, au Pakistan oriental, a montré dans 92% des cas une augmentation d'au moins trois fois (une dilution) du titre des anticorps neutralisant la toxine; dans 73% des cas, l'augmentation était de neuf fois (deux dilutions). Parmi 40 sujets chez lesquels les examens de selles étaient négatifs, on a noté à deux occasions une hausse des anticorps neutralisants. Bien que l'on ne puisse exclure ici un échec des techniques bactériologiques, ces deux observations sont considérées comme des réactions faussement positives.

Les anticorps neutralisant la toxine apparaissent précocement et la montée des titres sériques se produit généralement une semaine après le début de la maladie. Selon les auteurs, une augmentation de neuf fois ou plus des titres a la même sensibilité et la même spécificité pour le diagnostic rétrospectif du choléra qu'une hausse de quatre fois des titres d'anticorps vibriocides. De plus, comme elle ne se manifeste pas après administration du vaccin utilisé au Pakistan oriental, la valeur diagnostique de la méthode est supérieure à celle des autres réactions sérologiques.

Des titres relativement élevés d'anticorps neutralisant la toxine ont été décelés chez des enfants de moins de 15 ans dépourvus d'anticorps vibriocides ou agglutinants et sans antécédents d'infection à *V. cholerae*. De nouvelles recherches sont indispensables pour expliquer cette constatation assez paradoxale.

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