TABLE II. Formation Constants of Zinc Complexes of Various Complexing Agents by Ion-Exchange Method at a pH of 7.4.

Ligand	n	log formation constant $K_{f(1)}$	$K_{f(2)}$
Terephthalic acid	1	1.00	3.46
Lactic acid	1	1.60	1.90
Thiodipropionic acid	1	1.91	—
Sodium glycolate	1	1.94	2.24
" orthophosphate	1	2.22	1.66
Ethylenediaminebitartrate	1	2.60	1.60
Citric acid	1	4.53	—
Sodium acid pyrophosphate	1	5.27	4.93
" hexametaphosphate	1	5.56	5.46
" tripolyphosphate	1	5.79	4.81
Ethylenediaminediacetic acid (EDDA)	1	5.98	5.15
Hydroxyethylethylenediaminetriacetic acid (HEDTA)	1	6.00	7.40
1,2-Diaminecyclohexanetetraacetic acid (CDTA)	1	6.29	6.69
Dihydroxyethylenediaminediacetic acid (DHEEDA)	2	7.66	
Diethylenetriaminepentaacetic acid (DTPA)	2	12.27	
2-Hydroxypropylenediaminetetraacetic acid (HPDTA)	3	20.08	
Ethylenediamine-N, N'-diacetic acid-N, N'-dipropionic acid (EDDADP)	3	20.91	

No formation constant for zinc-phytic acid complex could be determined by this method because of its insolubility at a pH of 7.4. At this pH, glucose, fructose or lactose did not release Zn^{65} from the ion exchange resin. However, Zn^{65} was released if the pH of the solution was about 10.2.

Summary. Formation constants at a pH of 7.4 and $\mu = 0.16$ have been determined by the ion-exchange method for zinc and a number of complexing agents.

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A Study of Gamma Globulins in Cholera. (30795)

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The relationship of the various globulins to cholera has not been studied in man with the aid of recently developed methods of chromatographic and electrophoretic separation of serum proteins.

Cholera appears today as a disease caused either by the typical *Vibrio cholerae* or by its biotype, *V. cholerae El Tor*. The latter has been the agent of an epidemic in Asia that involved countries from Indonesia north to Japan and east to Iran since 1961. This so-called El Tor disease is attracting much attention. It seemed advisable, however, to first study "classic" cholera which predominated until 1961 in Asia, reaching out from time to time from its Indian cradle into other countries. The last "classic" cholera epidemic assailed Thailand in 1958 and was the subject of several studies. One of them(1) correlated the agglutinating, lethal toxin and El Tor hemolysin neutralizing potency of the sera of patients with cholera caused by different biotypes of *V. cholerae*. This communication is a continuation of those investigations, reporting the results of the comparison of agglutinating and cholera toxicity neutralizing antibodies with the changes in some im-

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munologically important fractions of the serum globulins.

Materials and methods. Sera. One hundred sixty-four sera were available from studies carried out between 1957 and 1961 in Japan and in Thailand. All were kept under refrigeration.

Ninety-two sera came from patients afflicted with cholera in Bangkok. The causative organism was *Vibrio cholerae,* Ogawa type. All persons excreted this vibrio in their stools. The course of disease was mild in all instances. Antibiotics were not given but replacement of the lost fluid and electrolytes was carried out. All patients were adults and had a history of having had received at least one injection of cholera vaccine within 6 months preceding the disease.

The sera were collected in intervals. Those drawn the first to third day of the illness were acute sera because in none of these instances did the malady last longer than 3 days.

Twenty control sera came from apparently healthy members of the Royal Thai Army in 1961. The soldiers were vaccinated every 6 months and were exposed in 1959 and 1960 to cholera that invaded their barracks but these individuals did not become clinically ill.

Fifty-two sera were available from a study carried out on Japanese nationals in 1957 in Tokyo. They received 0.5 and 1.0 ml of a commercial vaccine made in the United States and used by the U. S. Army in 7- to 8-day intervals. These persons were not exposed to cholera during the time of the experiment.

Methods. The Agafor electrophoretic apparatus (Agaton Co., Bern, Switzerland) was used. The sera were subjected to agar gel diffusion electrophoresis in 1% Nobel agar, on microscopic slides, for 2 hours at 2.25 V per cm. The buffer in the chamber was 0.1 veronal buffer, pH 8.6. Horse antihuman serum was employed. Amidoschwartz (Allied Chemical Corp.) was used to stain the slides.

The agglutination tests were carried out with a live culture of *V. cholerae* Ogawa type that was isolated from one of the patients whose serum was included in the series reported here. The serum dilutions were set up in duplicate, in halves of routinely used dilutions, *i.e.,* 1:40, 1:60, 1:80, 1:120, 1:160, etc. to 1:620. After 2 hours incubation in the water bath at 50 to 52°C, the tubes were kept in the biological refrigerator overnight. Only 3+ and 4+ results were registered as positive.

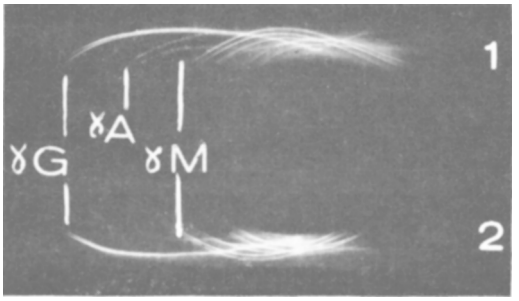

FIG. 1. Representative serum electrophoresis from two cholera patients in the convalescent (1) and in the acute stage (2) of their disease.

The cholera toxicity neutralizing capability of the sera was determined in the developing chick embryo according to the method described by Morgan *et al*(2).

Discussion. The results of these studies are shown in Table I. Virtually none of the cholera patients had only 7S gamma globulin. The majority had γ-G, A and M globulins even in the earliest phases of sampling. The γ-A arc appeared during the convalescence of most of the patients (Fig. 1). A stimulus to antibody production was noted by the presence of all 3 of the γ-globulin fractions in the sera of 12/18 of patients in the 3- to 6-month post-infection group. Thai controls were taken from a healthy group of soldiers. Their sera demonstrated a lesser quantity of the larger globulins. Japanese vaccinees responded with an increase in the γ-M globulins in the 4- to 28-day period following injection of cholera vaccine. The agglutinin titers and cholera toxigenicity neutralizing ability (CTNA) increased in the 4- to 10-day period following either infection or vaccination. The CTNA was significantly stronger in those patients whose antigenic stimulus was furnished by natural infection. A definite correlation between the γ-globulin pattern and the other immunologic features of the sera cannot be made. It appears that the response

TABLE I. Globulin Fractions, Agglutinin Titers and Cholera Toxigenicity Neutralizing Potency of 164 Sera.

Serum from	Time drawn	No. examined	7S only #Aggl/Antitox	7S and A #Aggl/Antitox	7S and M #Aggl/Antitox	7S, A and M #Aggl/Antitox
Patients	1– 3 days	22	1 0*/0†	2 0/0	18 0/0.4±0.2	1 0/0.6
	4–10 "	21	0	3 110±9/0.3±0.2	10 134±16/0.8±0.3	8 124±14/0.8±0.3
	11–28 "	16	0	4 188±14/2.0±0.6	3 158±14/4.5±0.8	9 172±20/5.6±1.1
	3– 6 mo	18	0	3 62±9/1.6±0.4	3 70±8/3.1±0.2	12 76±5/4.4±0.4
	12–18 "	15	2 0/0	9 0/0.8±0.3	2 0/1.3±0.4	2 0/0.9±0.5
Thai controls		20	10 0/0.4±0.2	3 0/0.6±0.2	5 0/0.6±0.2	2 0/1.1±0.3
Japanese vaccinees	Before	15	5 0/1	2 0/0	2 0/0	6 0/0
	4–10 days	15	0	1 80/0	10 71±9/0	4 86±7/0
	11–28 "	12	0	1 320/0.3	10 112±13/0.4±0.2	1 120/0.6
	3– 6 mo	10	2 0/0	4 66±12/0.4±0.1	2 72±30/0	4 61±23/0.3±0.2

= number 0* = less than 60 0† = less than 0.3

to cholera is similar to that seen with other bacterial diseases in spite of the unique pathophysiology of cholera. The large number of controls and of patients having γ-M-globulins prominent in their sera in the 1- to 3-day period of this study is probably due to the presence of concomitant and/or chronic disease in this segment of the Thai population. It is possible, however, that in the experimental group this increase was caused by an immediate sharp increase of this globulin.

Summary. The immunoelectrophoretic patterns of the gamma globulins were studied in the sera of Thai patients infected with *Vibrio cholerae,* Ogawa type. Normal Thai sera and sera obtained from Japanese vaccinees were used as controls. Relations were sought between the protein patterns, and the agglutinin titers as well as the cholera toxigenicity neutralizing ability (CTNA) of the sera. The immunologic response to cholera infection was similar to that in other bacterial diseases. A higher CTNA was noted in natural infection than after vaccination. As a group, the cholera patients had a high incidence of γ-M-globulin in sera taken in the acute phases of the disease. It is suggested that this might be due either to a rapid increase of this globulin or to the presence of concomitant and/or chronic disease.

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