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The serologic response of patients with bacillary dysentery (*Shigella dysenteriae* type 1) was tested by passive hemagglutination, and the results suggest that the test can become a useful adjunct in the diagnosis of this disease. Passive hemagglutination, using cell-wall O polysaccharide from various *Shigella,* proved to be highly specific for detection of antibodies in rabbit and human sera. Sera from 281 patients with bacillary dysentery had antibodies by the second day after onset of the disease. Maximal titers were reached by the eighth or ninth day. In half of the patients antibody titers fell to nonsignificant levels ($< 1:40$) by the fifth or sixth month. Antibodies to *S. dysenteriae* type 1 were sensitive to treatment with 2-mercaptoethanol. On the basis of this sensitivity, their hemagglutinating capacity, early appearance, and rapid catabolism, it appears probable that the antibodies to *S. dysenteriae* type 1 are in the IgM fraction of serum.

The recent regional epidemic outbreak of Shiga dysentery (*Shigella dysenteriae* type 1 infection) in Central America provided us with an opportunity to evaluate the specificity of the passive hemagglutination (PHA) test [1, 2] in detecting this infection. Our study describes the pattern of serologic response of patients infected with *S. dysenteriae* type 1 and contrasts it with that of patients infected with other shigellae.

Materials and Methods

Patients. Three groups of patients were studied: (*1*) 281 patients with severe clinical dysentery, who were seen from one to 30 days after onset, and most of whom had Shiga dysentery, as determined serologically; (*2*) 108 patients with bacteriologically confirmed bacillary dysentery due to various serotypes of *Shigella,* including the Shiga bacillus; and (*3*) 43 patients with clinical, bacteriologic, and serologic diagnoses of Shiga bacillus dysentery made three to nine months previously, who could be observed throughout this time span. Some of the cases in this group originally were in the series of 281 patients.

Monospecific sera. Monospecific sera were prepared by the conventional method outlined by Edwards and Ewing [3]. Immune sera were prepared against the epidemic strain of *S. dysenteriae* type 1, as well as against the serotypes that are commonly isolated in Central America, namely *S. dysenteriae* type 2, *S. flexneri* types 1, 3, 4, and 6, and *S. sonnei* [4–7].

Shigella O polysaccharide. Crude O polysaccharide antigens of *Shigella,* corresponding to the above serotypes, were prepared by the method of Young et al. [8, 9].

Passive microhemagglutination. Passive microhemagglutination [10] with formalin-preserved erythrocytes [11, 12] was employed. Agreement between duplicate measurements was better than 94% [13]. Antibody titers $\geqslant 1:40$ were considered indicative of active or recent infection [14].

Treatment with 2-mercaptoethanol. Sensitivity of antibody to 2-mercaptoethanol was determined by incubation of equal volumes of undiluted serum and 0.4 M 2-mercaptoethanol (*p*H 7.2) for 5 hr at 4 C in the dark. Treated sera were tested in

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duplicate and compared with nontreated sera examined on the same day.

Results

Specificity of the hemagglutination reaction. Passive microhemagglutination of each O polysaccharide with monospecific rabbit antisera showed a high specificity for homologous reactions with *S. dysenteriae* types 1 and 2 and *S. sonnei* (table 1). The O polysaccharide of other *Shigella* also reacted, but titers for the homologous system were four- to eightfold higher than for the heterologous system. Similar results were obtained with 108 sera from patients with shigellosis of various serotypes, predominantly *S. dysenteriae* 1; only one (1.5%) showed significant cross-reaction with the Shiga bacillus (table 2).

Serologic response to S. dysenteriae type 1. In individuals with *S. dysenteriae* type 1 or 2 whose blood could be examined three to five days after onset and on following days, antibodies were evident promptly after infection and rose to high titers during the first and second week after onset. Of 281 patients with clinically manifest Shiga dysentery who could be examined at various periods after onset, antibodies were demonstrated on the second day (figure 1) in at least one of seven. By the fourth day, six of 15 patients had detectable antibodies. After the ninth day, more than 80% of patients had significant titers of antibody to the Shiga bacillus.

In the group of 43 patients who had had Shiga dysentery within the preceding three to nine months, 80% had positive serologic reactions one month after their illness. Later, there was a progressive decline, so that by the third month, about one-half of the sera had significant titers of antibody, and by the ninth month, only 20% had such titers (figure 1).

Table 2. Specificity of sera from patients with shigellosis due to *Shigella dysenteriae* type 1 and to other *Shigella.*

Shigella isolated	No. of positive patients*	Significant antibody titer (1:40)	
		Homologous	Heterologous
S. dysenteriae 1	70	33 (44.3)†	1 (1.5)
Other‡	38	16 (42.1)	6 (15.8)
Total	108	47 (43.5)	7 (6.5)

* Confirmed by isolation of the organism.
† Number of cases with significant titer (percentage).
‡ Includes *S. dysenteriae* type 2, *Shigella flexneri* types 1 and 6, and *Shigella sonnei.*

Titers of antibody to the Shiga bacillus were highest in the first week of the disease (geometric mean titer, 1:480). The mean antibody titer also decreased with time, to a low value after six months (figure 2).

Sensitivity to 2-mercaptoethanol. Sera previously positive showed no significant titers after treatment with 2-mercaptoethanol regardless of the initial value.

Discussion

Hemagglutinating antibodies to the Shiga bacillus could be detected on the first few days of infection, an observation that holds also for other Enterobacteriaceae [15–17]. By the eighth or ninth day, 80% of the patients had significant antibody titers that reached maximal levels after one month. Nevertheless, by the seventh month after onset, few patients had significant levels of antibody. A similar pattern has been reported for other *Shigella* [18, 19].

The humoral immune response to gastrointes-

Table 1. Specificity of HA reactions with antigens and monospecific antisera to *Shigella.*

Shigella antigen	Monospecific antisera						
	a	b	c	d	e	f	g
A *dysenteriae* 1	**2,560***	0†	0	0	0	0	0
B *dysenteriae* 2	0	**2,560**	0	0	0	0	0
C *flexneri* 1a	0	0	**10,240**	0	0	40	0
D *flexneri* 3	0	0	0	**2,560**	40	0	0
E *flexneri* 4	0	0	0	160	**1,280**	0	0
F *flexneri* 6	0	0	160	0	0	**10,240**	0
G *sonnei*	0	0	0	0	0	0	**2,560**

* Numbers are reciprocal titers; boldface numbers indicate homologous reactions.
† 0 = Negative in HA test at 1:40 dilution.

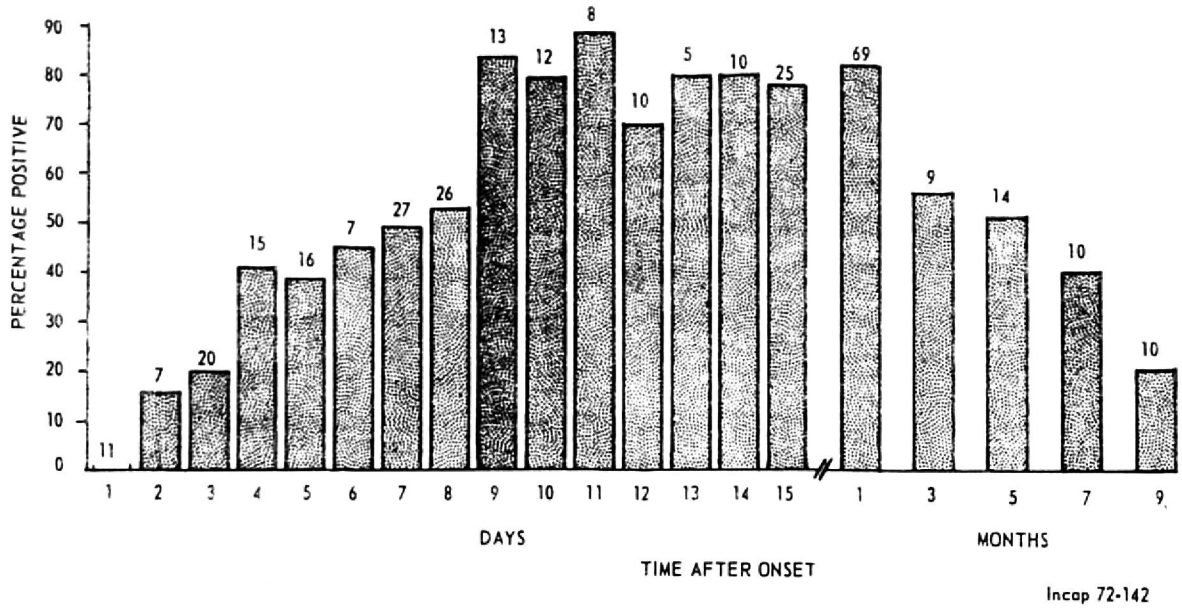

Figure 1. Percentage frequency of patients with significant titers of passive HA antibody to the Shiga bacillus among 281 patients with severe bacillary dysentery studied at various periods in the first two weeks and among 43 patients with proven Shiga dysentery, three to nine months later.

tinal infections depends on the intensity of invasion of the gastrointestinal tract and the extent of damage to the intestinal mucosa [20]. Patients with Shiga bacillus dysentery had higher titers of antibody than patients with bacillary dysentery other than the Shiga type [21, 22]. Antibody titers of patients in our study also were greater than those reported for people vaccinated orally with killed shigellae [23].

Several facts suggest that the antibody we studied belonged in the IgM fraction. Its titer rose quite rapidly, and it declined at a relatively rapid rate; its activity was abolished by treatment with 2-mercaptoethanol. Moreover, its activity was directed against the O polysaccharide, and several studies on experimental animals [24, 25], children vaccinated with inactivated enteric bacteria [26, 27], and patients with natural infections due to salmonellae [28, 29] have shown that antibodies against enterobacterial O polysaccharide were of the IgM type. Proof of this supposition must await studies by chromatography, ultracentrifugation, and immunochemistry. The passive HA test has proved useful for clinical diagnosis and epidemiologic studies of enteric infections [30–32]. The study showed it to be reliable in the specific diagnosis of Shiga dysentery; 80% of the patients showed significant titers of antibody in the first few days after onset of clinical disease in comparison with similar reported determinations in other shigelloses [21, 32, 33]. Tests repeated several days later confirmed a rise in antibody titers. This serologic test can be a useful adjunct in clinical diagnosis of Shiga bacillus dysentery, but it should not replace isolation of the specific bacillus. On the other hand, this test can be used to great advantage in public health surveillance programs. [34].

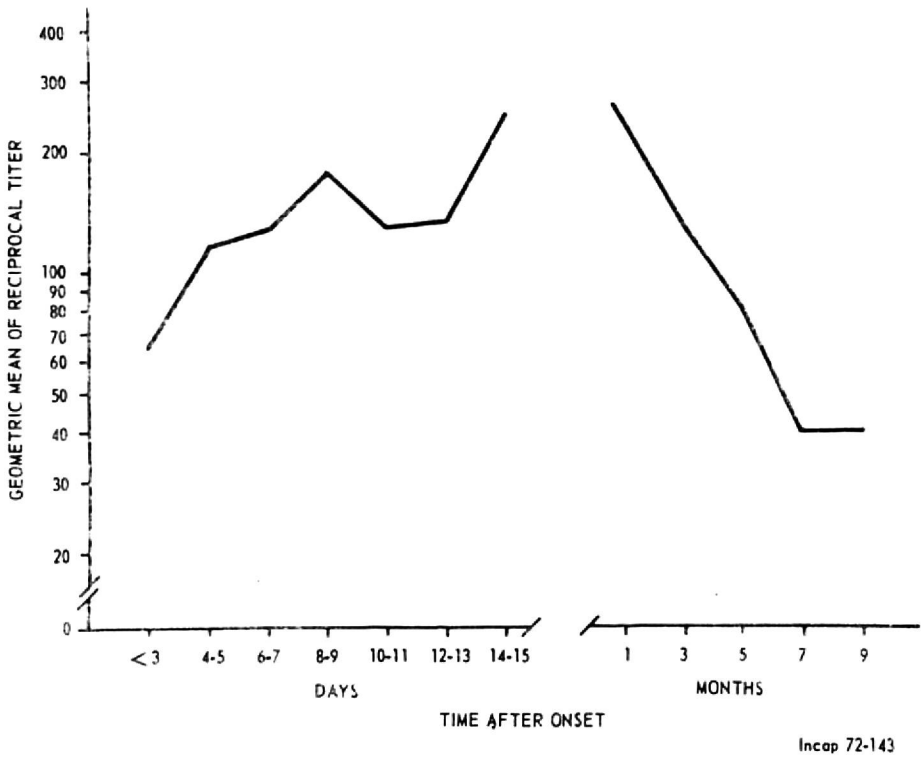

Figure 2. Geometric mean of passive HA antibody titers to Shiga bacillus. The left side of the figure refers to the cases among the series of 281 patients, and the right side refers to the 43 cases observed from three to nine months after onset.

The potential usefulness of this test in the diagnosis of dysentery should not becloud the fact that circulating antibodies probably are of little importance in the elimination of infectious agents from the gastrointestinal tract [35–38]. This protection derives mainly from the action of secretory IgA and mechanisms of cellular immunity [39, 43].

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