

ENTEROPATHOGENICITY OF *AEROMONAS HYDROPHILA* AND *PLESIOMONAS SHIGELLOIDES*

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PLATE XI

AEROMONAS HYDROPHILA and *Plesiomonas shigelloides* have been isolated from stools of children and adults with diarrhoea of otherwise unexplained origin (von Graevenitz and Mensch, 1968; Sakazaki *et al.*, 1971; Chatterjee and Neogy, 1972; Sanyal *et al.*, 1972a and b). These reports indicate the possible enteropathogenicity of *A. hydrophila* and of *P. shigelloides*: they were the dominant microbial flora in some patients with diarrhoea and were isolated more often from patients than from symptomless persons; but no experimental evidence has been reported in support of their enteropathogenicity. In recent years many of the diarrhoeagenic organisms such as enteropathogenic *Escherichia coli*, *Shigella* spp., *Clostridium perfringens* (*welchii*), and *Vibrio parahaemolyticus* have, like *V. cholerae*, been found to cause accumulation of fluid in the adult-rabbit ileal loop (De, Bhattacharya and Sarkar, 1956; Duncan and Strong, 1969; Keusch, Mata and Grady, 1970; Sakazaki *et al.*, 1974). Because *A. hydrophila* and *P. shigelloides* have been associated with diarrhoea, the present study was made to examine their enteropathogenicity in this experimental model.

MATERIALS AND METHODS

Organisms. The strains of *A. hydrophila* and *P. shigelloides* used in this study (tables I and II) were isolated by us from children and adults with diarrhoea, healthy individuals, potable water, and the river Ganges. They were characterised by the method of Hugh (1970). The toxigenic *V. cholerae* strain no. 11-A was isolated in this laboratory from a patient with cholera and consistently gave a positive reaction in the ileal loop. The organisms were maintained in stab cultures and did not undergo more than three subcultures.

Pathogenicity test. This was performed in adult-rabbit ileal loops by the method of De and Chatterjee (1953). Rabbits weighing 1.5–2.0 kg were starved for 24 h with only water to drink. The intestines were exposed by laparotomy; beginning near the ileocaecal junction, loops 10-cm long were tied with an interval of approximately 5 cm between each. Usually six loops were made in one rabbit. Into each loop the test organism was inoculated as 1 ml of a 10^{-3} saline dilution from a 3–4-h nutrient-broth culture, containing about 10^5 bacterial cells. The first loop always served as a positive control with a toxigenic *V. cholerae* strain and the last as a negative control with isotonic saline. Each strain was tested in 2–10 rabbits. The animals were killed after 16–18 h and the reactions in the loops noted. The length of each loop and the volume of fluid in it were measured to determine the fluid collection per unit length. If there was any fluid in any of the gut between loops or any negative reaction in the positive-control loops, the results were discarded.

Multiplication of bacteria in ileal loops. A viable count of each inoculum was made in duplicate by a pour-plate method. At laparotomy the fluid was collected aseptically and a viable count was made as above. Different colony types—which were usually not more than two at lower dilutions and only one at higher dilutions (10^5 or 10^6)—were counted on the duplicate plates and tested biochemically for identification. The mean count multiplied by the volume of fluid gave the total number of bacteria in each loop. In cultures from positive

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loops only one colony type was usually noted. When there was no reaction, fluid for the viable count was obtained by removing the loop and repeatedly washing the lumen with 10 ml of sterile isotonic saline.

RESULTS

Of the 14 strains of *A. hydrophila*, 12 repeatedly gave a positive ileal-loop reaction, nine of them doing so in all the experiments (table I). The two strains from non-diarrhoeal individuals and the one from Ganges water were positive. Strain no. 150, from water, showed a gut-inflammatory reaction in 9 of the 10 loops tested. Two strains gave negative reactions: one came from a diarrhoeal and the other from a non-diarrhoeal adult. The fluid accumulation ranged from 1.0 to 2.0 ml per cm for *A. hydrophila* test strains and from 1.0 to 1.8 ml for *V. cholerae*. All the positive strains except one showed fluid accumulation of 1.2 ml or more per cm of gut length. The reactions by both the groups of organisms and the positive and negative controls are shown in the plate.

P. shigelloides, on the other hand, yielded very few positive results (table II). Some of the strains showed small fluid accumulation occasionally. Strain no. 3869 caused a small amount of haemorrhagic fluid in one loop (0.2 ml per cm).

Two strains of *A. hydrophila* (Suchi and no. 150) and two strains of *P. shigelloides* (nos. 3186 and 292) were tested for multiplication in ileal loops; three experiments were performed with each strain. Both strains multiplied by about 10^5 whereas the *P. shigelloides* did so only by 10^{2-3} (table III).

DISCUSSION

For *A. hydrophila* the results indicate high pathogenicity of the organisms irrespective of their sources. Accumulation of fluid in the loops was up to 2 ml per cm, compared with 1.0-1.8 ml per cm for the toxigenic *V. cholerae*. This suggests high toxigenicity of some of the strains. It was also observed that when higher dilutions were used as inocula, even 10^4 viable bacteria could produce a gut reaction.

The strains of *P. shigelloides* showed little evidence of causing reaction each when tested in 2-8 loops. In a few cases there was negligible fluid accumulation. While looking for an explanation for non-reactivity of these organisms we observed that their rates of multiplication

TABLE I
Enteropathogenicity test for Aeromonas hydrophila in rabbit ileal loop

Strain number or designation	Source	Number of positive reactions/number of tests	Accumulated fluid: range in ml per cm of gut
2724	Diarrhoeal child	6/6	1.2-1.8
Pappu	Diarrhoeal child	3/4	1.2-1.7
1470	Diarrhoeal child	3/3	1.3-1.5
2741	Diarrhoeal child	3/3	1.4-2.0
2884	Diarrhoeal child	3/3	1.2-1.8
4511	Diarrhoeal child	3/3	1.5-2.0
Suchi	Diarrhoeal child	5/5	1.5-2.0
4130	Non-diarrhoeal child	3/3	1.3-1.6
4006	Non-diarrhoeal child	3/3	1.0-1.8
4138	Non-diarrhoeal child	0/2	Nil
4171	Diarrhoeal adult	3/4	1.2-1.4
1133	Diarrhoeal adult	0/2	Nil
150	Water	9/10	1.6-2.0
81	Ganges water	3/3	1.4-1.8
<i>V. cholerae</i> 11-A	Positive control	18/18	1.0-1.8
...	...	0/18	Nil
	(Negative control)		

TABLE II
Enteropathogenicity test for Plesiomonas shigelloides in rabbit ileal loop

Strain number	Source	Number of positive reactions/number of tests	Accumulated fluid: ml per cm of gut
517	Diarrhoeal child	0/3	Nil
3186	Diarrhoeal child	1/8	0.30
292	Diarrhoeal child	1/8	0.30
541	Diarrhoeal child	1/6	0.15
3869	Non-diarrhoeal adult	1/2	0.20
136	Ganges water	0/2	Nil

in rabbit ileal loop were much lower than that of *A. hydrophila*. The ability of the potentially enterotoxigenic bacteria to multiply freely in the intestine of the rabbit is possibly an essential factor governing the accumulation of fluid in the ileal loop. Enterotoxigenic substances produced by the organism during multiplication are presumably responsible for inducing fluid outpouring. These experiments indicate that tests of fluid collection in the rabbit ileal-loop model cannot confirm the ability of *P. shigelloides* to produce enterotoxin.

The present study clearly established the enteropathogenic nature of *A. hydrophila* in the rabbit gut-loop model, which has been found to give highly reproducible in studies results on other diarrhoeagenic bacteria that are known to produce an enterotoxin. No definite conclusion, however, could be drawn from these experiments on the enteropathogenicity of *P. shigelloides*.

SUMMARY

Aeromonas hydrophila was enteropathogenic in ligated ileal loops of rabbits, causing a fluid accumulation of 1.0–2.0 ml per cm of gut length. Gut reaction could be produced with an inoculum as low as 10^4 viable bacteria. There was no difference in the nature of the positive reactions given by strains isolated from diarrhoeal and non-diarrhoeal children and adults and from water. *Plesiomonas shigelloides*, on the other hand, did not cause a significant gut reaction. *A. hydrophila* multiplied in the ileal loop by about 10^5 whereas *P. shigelloides* did so at only 10^{2-3} . These experiments on an animal model thus indicated the enteropathogenic nature of *A. hydrophila*, but no definite conclusion could be drawn from this study on *P. shigelloides*.

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TABLE III
Multiplication of Aeromonas hydrophila and Plesiomonas shigelloides in rabbit ileal loop

Strain number or designation	Number of experiments	Size of inoculum	Final viable count
<i>A. hydrophila</i> (Suchi)	3	3.75×10^5	8.63×10^{10}
<i>A. hydrophila</i> (150)	3	2.34×10^4	5.26×10^9
<i>P. shigelloides</i> (3186)	3	2.75×10^5	3.25×10^7
<i>P. shigelloides</i> (292)	3	3.20×10^4	3.42×10^7

REFERENCES

- CHATTERJEE, B. D. AND NEOGY, K. N. 1972. On the aetiology of choleraic diarrhoea. *Indian J. med. Res.*, **60**, 531.
- DE, S. N., BHATTACHARYA, K. AND SARKAR, J. K. 1956. A study of the pathogenicity of strains of *Bacterium coli* from acute and chronic enteritis. *J. Path. Bact.*, **71**, 201.
- DE, S. N. AND CHATTERJEE, D. N. 1953. An experimental study of the mechanism of action of *Vibrio cholerae* on the intestinal mucous membrane. *J. Path. Bact.*, **66**, 559.
- DUNCAN, C. L. AND STRONG, D. H. 1969. Ileal loop fluid accumulation and production of diarrhoea in rabbits by cell-free products of *Clostridium perfringens*. *J. Bact.*, **100**, 86.
- GRAEVENITZ, A. VON AND MENSCH, A. H. 1968. The genus *Aeromonas* in human bacteriology. *New Eng. J. Med.*, **278**, 245.
- HUGH, R. 1970. *Pseudomonas* and *Aeromonas*. In Manual of clinical microbiology, edited by J. E. Blair, E. H. Lennette and J. P. Truant. *Bethesda, Md.*, p. 175.
- KEUSCH, G. T., MATA, L. J. AND GRADY, G. F. 1970. Shigella enterotoxin: isolation and characterization. *Clin. Res.*, **18**, 442.
- SAKAZAKI, R., TAMURA, K., NAKAMURA, A., KURATA, T., GOHDA, A. AND KAZUNO, Y. 1974. Enteropathogenicity of *Vibrio parahaemolyticus*. In International Symposium on *Vibrio parahaemolyticus*, Tokyo, p. 231.
- SAKAZAKI, R., TAMURA, K., PRESCOTT, L. M., BENCIC, Z., SANYAL, S. C. AND SINHA, R. 1971. Bacteriological examination of diarrhoeal stools in Calcutta. *Indian J. med. Res.*, **59**, 1025.
- SANYAL, S. C., GAUR, S. D., SHRIVASTAVA, D. L., SEN, P. C., MARWAH, S. M. AND HARDAS SINGH 1972a. Enteric infections in Sunderpur slum area. *Indian J. med. Res.*, **60**, 979.
- SANYAL, S. C., SEN, P. C., CHAKRABARTY, D. P. AND HARDAS SINGH 1972b. Bacteriological investigations of gastroenteritis amongst Bangladesh refugees. *Indian J. prev. soc. Med.*, **3**, 183.

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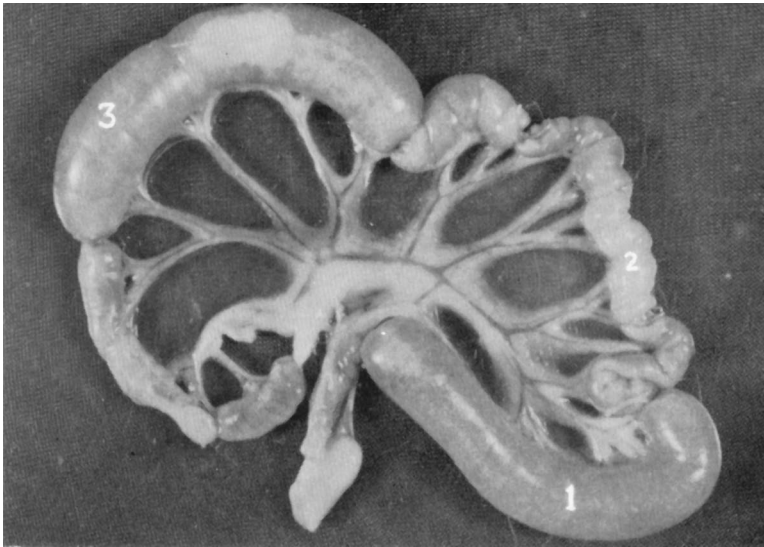


FIGURE.—Loop 1: reaction with the control strain of *Vibrio cholerae*; loop 2: no fluid collection with *Plesiomonas shigelloides*; loop 3: positive reaction with *Aeromonas hydrophila* showing marked fluid accumulation.