

De S N & Chatterje D N. An experimental study of the mechanism of action of *Vibrio cholerae* on the intestinal mucous membrane. *J. Pathol. Bacteriol.* 66:559-62, 1953.  
[Department of Pathology, Nilratan Sircar Medical College, Calcutta, India]

Injection of living *Vibrio cholerae* into the lumen of a loop of rabbit intestine isolated by a ligature causes accumulation within this loop of a large amount of fluid having gross similarity with cholera stool. Evans blue solution injected intravenously leaks into this fluid suggesting alteration of permeability of intestinal capillaries by *Vibrio cholerae*. [The SC® indicates that this paper has been cited in over 370 publications since 1955.]

J.K. Sarkar  
87, Golf Club Road  
Calcutta 700 031  
and  
A. Sen  
L-5, Vidyasagar Niketan  
Salt Lake Sector 1  
Calcutta 700 064  
India

December 16, 1986

While working in the laboratory of Sir Roy Cameron at the University Medical School in London on the pathology of experimental hydrocephalus, De developed a keen interest in experimental bacteriological pathology as he watched the course of another study, this one on Shiga toxin, being carried on in the same laboratory. He must have conceived the idea of working on cholera at that time, as he brought with him from London appliances for experimenting with rabbits. Immediately on his return to Calcutta, even before he became professor of pathology at Nilratan Sircar Medical College, De started experimental work on the pathogenesis of cholera with his former colleagues at the Calcutta Medical College.<sup>1</sup> He did not believe in the poison theory of Koch.

First, De attempted to produce symptoms of cholera in rabbits by injecting heavy cultures of *V.*

*cholerae* into the small intestine. The animals subsequently had no diarrhoea, but on autopsy the huge caecum, which normally contains pasty semisolid material, was found to be distended with semiliquid faecal matter from which *V. cholerae* could be recovered. De argued that the fluid that poured into the small intestine accumulated in the caecal backwater and could not find its way out. So next, he bypassed the caecum, isolated a four-inch segment of small intestine by silk ligatures, introduced *V. cholerae* mixed with 1 ml peptone water medium, and killed the animals the next day. The ligated segment was distended with about 15 ml rice-water fluid while the control segment was collapsed. Evans blue solution injected intravenously leaked into this fluid suggesting alteration of permeability of intestinal capillaries.

Thus De found that he had a satisfactory animal model for studying the effect of *V. cholerae* and other enteropathogenic organisms. He studied the pathogenicity of strains of *E. coli* from acute and chronic enteritis by this technique.<sup>2</sup> The success of this animal model probably led to extensive citation of the work.

At Calcutta Medical College, which De had joined in 1956, facilities for further studies were not available, and consequently he also worked at the Bose Institute where one of us (AS) was his colleague. In subsequent studies he showed, by injecting bacteria-free culture filtrate into the isolated rabbit loops, that the loops were distended with fluid and thus proved the enterotoxicity of the culture filtrate.<sup>3</sup> Finally, he established the absence of any role of the endotoxin in the outpouring of fluid by the negative results he obtained from injecting sterile extracts of sonicated, washed vibrios into rabbit loops. He concluded that it was a cholera exotoxin that caused fluid accumulation in the small intestine.<sup>4</sup> De's work on cholera exotoxin has recently been reviewed.<sup>5,6</sup>

Till the end of his life, De believed that (1) a classical enterotoxin serotype is not necessarily pathogenic, and (2) for testing enteropathogenicity, strains of *E. coli* or *V. cholerae* should be freshly prepared.<sup>7</sup>

De's integrity and high sense of dignity held him aloof from the powers that control awards, honours, and fellowships, but many in his own field or related disciplines knew the merit of his contribution.

1. De S N, Sarkar J K & Tribedi B P. An experimental study of the action of cholera toxin. *J. Pathol. Bacteriol.* 63:707-17, 1951. (Cited 10 times since 1955.)
2. De S N, Bhattacharya K & Sarkar J K. A study of the pathogenicity of strains of *Bacterium coli* from acute and chronic enteritis. *J. Pathol. Bacteriol.* 71:201-9, 1956. (Cited 125 times.)
3. De S N. Enterotoxicity of bacteria-free culture-filtrate of *Vibrio cholerae*. *Nature* 183:1533-4, 1959. (Cited 110 times.)
4. De S N, Ghose M L & Sen A. Activities of bacteria-free preparations from *Vibrio cholerae*. *J. Pathol. Bacteriol.* 79:373-80, 1960. (Cited 45 times.)
5. van Heyningen W E & Seal J R. *Cholera: the American scientific experience, 1947-1980*. Boulder, CO: Westview Press, 1983. p. 60.
6. Garfield E. Mapping cholera research and the impact of Shambu Nath De of Calcutta. *Current Contents* (14):3-11, 7 April 1986.
7. De S N. The value of serotyping of *E. coli* isolated from cases of acute diarrhea. (Ouchterlony O & Holmgren J, eds.) *Cholera and related diarrheas: molecular aspects of a global health problem*. New York: Karger, 1980. p. 222.