Assay of Enterotoxin in Animal Models*

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Animal models are important in studying infectious diseases for determining with certainty the microbial etiology of the disease. Assay of enterotoxin has been largely dependant on animal models. With animal models the pathogenesis of infectious diseases can be understood and immunization procedure can be established. Initial [illegible] with animal models on [illegible] use of pharmacological agent for therapy open the doorway for better treatment in humans.

Various animal models have been desribed following Koch's description of *Vibrio coma* now called *Vibrio cholerae*.[1,2] All the early studies in animal models were carried out with live organism challenges. In this paper I do not want to go to the review of those models. I am also not going to discuss the animal models that have been used for mouse protection test or test for vascular permeability in the skin of the back of adult guinea pigs and rabbits, food pad of rats and mice or other models where the model is not directly concerned with the assay of the material in the mammalian small intestine.

I would like to divide the discussion into two parts based on whether the animal has been used without any surgery as intact host model or based on a surgical process resulting in ligated loop or fistula in the small intestine.

About 20 years ago the infant rabbit model was described by Dutta and Habbu.[3] Oral inoculation of live culture of cholera vibrios were made and the animals produced diarrhoea. Similarly when enterotoxin is introduced into the stomach by a tube the animals come down with diarrhoea. I have carried out this technique in our laboratory and found that two preparations of cholera toxin and the cholera vibrios themselves produce the diarrheal response. I also observed in one out of several strains of NAG vibrios producing diarrhoea. Other NAG vibrios give negative results. It should be mentioned here that the infant rabbits become susceptible with age and become completely resistant when they are one month or more older. Infant rabbit model has been used for the assay of the toxin.

Intact host model for dogs[4] work better with live cholera vibrio challenge rather than the cell free enterotoxin. In both cases with appropriate dosage diarrhoea can be produced. Dog being a larger animal this model has been preferred for quite a number of patho-physiological studies. Immunologic studies including efficacy of vaccines, rechallenge experiment and chronic carrier state has been carried out in this model.

Intact host model with monkeys also produced diarrhoea in monkeys. We carried out several experiments in our laboratory where both live cholera vibrio and cholera toxin were used as inoculum introduced to the stomach by a tube. In both the cases the monkeys came down with diarrhoea. Guinea pigs, rats and mice were also used for producing cholera infection.[5,6]

Ligated ileal loops in adult rabbits have been very extensively used for the assay of cholera toxins.[7,8,9] This assay method directly measures the fluid accumulated in the small intestine. We described a rat model for the assay of cholera toxin by using the small intestine of rats.[9] Subsequently, guinea pig, mice and other animals were shown to produce fluid accumulation in the loop when challenged with enterotoxins.[8,10] Despite these subsequent discoveries rabbit ileal loop model still today is the most widely used model for the assay of cholera toxin. Canine intestinal loop was first used for studying the effect of cholera toxin on water and ion fluxes by Swallow *et al.*[11] in 1965. Subsequently, Thiryvella loops were used by many investigators.[12,13,14,15,16] Thiryvella loops consistently produced fluid and rechallenge experiments were carried out with this model by Sack and Carpenter in 1969.[17,18,19] Dose response curve could be established with varying doses of cholera toxin but the dose required for minimal response was at least 100 fold more than the amount of toxin required by rabbit small intestine loops.

On several occasions I have used rhesus monkey to test the response in the small intestine.[21] As in the case of dogs it took a 100 times more toxin to get a consistent response of fluid accumulation. In the case of monkeys I used loops open at both ends and was able to monitor fluid accumulation on a continual basis.

As a result of the animal studies we may have some understanding about pathophysiology of cholera.

Many investigators including myself have tried quite a number of pharmacologically active compounds but no compound that can be used as a drug has been able to stop the fluid accumulation once the gut is exposed to cholera toxin. Available vaccines have so far not proven to be of value as a worthwhile public health measure to protect a community against cholera. However, as we all know, nobody need die of cholera, if proper treatment is available to him. The current status of understanding about cholera, *E. coli* diarrhoea and abigellosis would not be possible if appropriate animal model would not be available for laboratory studies.

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