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This Week's Citation Classic

Dean A G, Ching Y-C, Williams R G & Harden L B. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. *J. Infec. Dis.* 125:407-11, 1972.
[Pacific Res. Sect., Natl. Inst. Allergy and Infectious Dis., Natl. Insts. Health; Dept. Pediat., Kaiser Med. Ctr.; and Dept. Pediat., US Army Tripler Gen. Hosp., Honolulu, HI]

This paper described a new test for *Escherichia coli* enterotoxin, based on intragastric inoculation of infant mice with culture supernates. Fluid accumulation in the gut was used as an index of toxin production. The toxin was found to be heat stable. A study of 37 Honolulu children with diarrheal disease disclosed no heat-stable enterotoxin (ST)-producing *E. coli*, in contrast to reported studies in India.[1] [The SCI® indicates that this paper has been cited in over 455 publications since 1972.]

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"The rabbit loop test for cholera and *E. coli* enterotoxin (heat labile or LT toxin), described by De *et al.* in 1956,[2] was a useful test. But it required anesthesia, laparotomy, tying off intestinal segments, and an overnight wait. At times, only half the rabbits responded properly and the test had to be repeated.

"In 1970, I was using the rabbit test to search for causative agents of diarrhea in specimens from seasonal diarrhea outbreaks in the Philippines. The screening of adequate numbers of specimens for enterotoxigenic *E. coli* with the rabbit test was a severe drain on available resources, and, while continuing the daily rabbit surgery, I began experiments to develop a better method. Baby chicks were cheaper than rabbits, but the gut was too short for more than a few tests, and the surgery no easier. Frogs, adult rats, and mice had the same drawbacks. Mosquito larvae swam happily in the toxin without swelling or shrinking. *In vivo* segments of extirpated mouse intestine failed to react to the toxin.

"Baby mice, on the other hand, responded nicely to culture supernates given by polyethylene esophageal tube, but getting the tube down without ripping the esophagus required delicate handling. The milk-filled stomach, visible through the translucent body wall of neonatal mice, offered a convenient target for direct intragastric inoculation.

"Feeling sure that the ensuing peritonitis would negate the results, I ventured a few mice to find out. Peritonitis aside, the results at four hours were beautiful, and the toxin turned the intestine into a tiny glistening bicycle tire full of fluid. It was obvious that the mouse intestine was responding to something different from cholera toxin, which is heat stable, since brief boiling failed to inactivate culture filtrates, and cholera toxin gave only a very weak response. Refinements were added over the next several months, such as weighing the gut, calculating the gut-to-carcass ratio, adding dye to the inoculum to document success of the injection, and experimenting with timing and various culture media. The result was a much more convenient test than the rabbit intestinal segment method.

"In collaboration with the clinical coauthors of the paper, Ching, Williams, and Harden, the stools of 37 Honolulu children with diarrheal disease were analyzed for ST-producing *E. coli*, with negative results. Subsequent studies have confirmed that ST strains are rare in the US, but relatively common in developing countries.

"In 1971, it seemed likely that someone would develop a simpler, more convenient test, based on biochemical or immunologic techniques, within months. This was not the case, however, and it has taken a decade of effort by many investigators to discover that human ST is a small molecule composed of 18 amino acid residues with important disulfide linkages.[3]

"The test has been used for detection of ST in clinical, epidemiologic, and laboratory research and is therefore cited frequently. By attaching ST to a carrier molecule, specific neutralizing antibody has been prepared, and a radioimmunoassay for ST has now been described.[4] Recently, Klipstein *et al.*[5] have reported synthesizing a molecule which has almost identical biological activity to ST. The production of ST by *E. coli* is under plasmid control,[6] and it seems that all the crucial facts are in place for further remarkable advances in understanding the exact mechanisms of production and action of ST and for development of a vaccine and/or effective method of treatment for ST-related diarrheal disease."

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3. **Staples S J, Asher S E & Giannella R A.** Purification and characterization of heat-stable enterotoxin produced by a strain of *E. coli* pathogenic for man. *J. Biol. Chem.* 255:4716-21, 1980.
4. **Frantz J C & Robertson D C.** Immunological properties of *Escherichia coli* heat-stable enterotoxins: development of a radioimmunoassay specific for heat-stable enterotoxins with suckling mouse activity. *Infec. Immunity* 33:193-8, 1981.
5. **Klipstein F A, Engert R F & Houghten R A.** Properties of synthetically produced *Escherichia coli* heat-stable enterotoxin. *Infec. Immunity* 39:117-21, 1983.
6. **Willshaw G A, Smith H R & Rowe B.** Cloning of regions encoding colonisation factor antigen 1 and heat-stable enterotoxin in *Escherichia coli*. *FEMS Microbiol. Lett.* 16:101-6, 1983.