# Effect of fasting and *Escherichia coli* heat-stable (S T) toxin on intestinal epithelial cell counts in rats

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## INTRODUCTION

Because of the intimate relationship between the intestinal cells and ingested foods, it is expected that food may play a role an regulating intestinal epithelial cell renewal (1). This study aims to compare the crypt and villous epithelial cell counts in upper small intestines of healthy suckling Wistar rats, during fasting states, and during a state of toxic secretory response to enterotoxigenic E. coli. (ETEC) heat-stable (ST) toxin.

## MATERIALS AND METHODS

Two - to four-day old suckling Wistar rats weighing four to six grammes were obtained from the Animal Services Division. That suckling Wistar rats were responsive to ST-toxin of ETEC had been demonstrated in a previous study (2). A known strain (C175/83 W12-3) of ST-producing ETEC isolate was obtained from the Bacteriology Research Division; crude ST-toxin was obtained by inoculating this strain in 1.5 ml trypticase soy broth kept under vigorous agitation at 37 degree centigrate overnight on a New Brounswick Model G2 roller shaker, then centrifuge at 10,000 rpm (12,000-17,000 g) for 30 minutes, and the supernatant fluid was used for intragastric injection in rats. In experiment 1, rats were given intragastric injection of either ST-toxin containing medium or control (not inoculated with E. coli.) medium. In experiment 2, two control rats were injected intragastrically with control medium, twelve rats were fasted (food and water was not given) at room temperature for 6 hours after which 6 rats were injected with control medium and 6 rats with medium containing ST-toxin of ETEC. Experiment 3 was similar to experiment 2 except that the fasting time was prolonged to 24 hours.

The response to toxin or control medium was assessed using the method described by Giannella (3), with the modification that rats were killed with a sharp blow on the head instead of anaesthesia by chloroform since physiological changes in the gut may occur in response to chloroform. An analytical balance sensative to $\pm$0.001 gramme was used. Gut-to-carcass weight ratios >0.085 were taken as a positive secretory response (3).

From each rat, small intestinal sections were taken and slides made. Three slides (each containing 9 to 10 transverse sections) were selected for cell counting using an Olympus microscope (Model P220.240); only those containing axially-sectioned crypts and villi,(i.e, base, middle and tip of villi

and crypt were all in the plane of the sections) were selected for counting. The left-hand column cells were counted 3 times, and only when 2 counts or more tallied were the counts recorded for each villi or crypt columns. This counting process was repeated for 3 slides per rat.

## RESULTS

With 6-hour fasting, the number of crypt cells were reduced from 5.83 (SE ±0.39) to 4.33 (SE ±0.31) which was statistically significant ($P < 0.01$); this was also accompanied by a significantly ($P < 0.01$) reduced crypt-villous ratios from 0.14 (SE ±0.01) in control rats to 0.10 (SE ±0.01) in fasted rats. No significant changes were observed with 24 hour fasting alone or during toxic secretory state following injection of ST-toxin of ETEC. Although cell counts were not significantly altered during toxic secretory state in response to ST-toxin after 6-hour fasting, crypt-villous ratio was significantly ($P < 0.01$) increased to 0.18 (SE ±0.01). During toxic secretory response to ST-toxin after 24-hour fasting, crypt cell counts were increased significantly ($P < 0.01$) to 7.33 (SE ±0.42), villi counts were reduced significantly ($P < 0.02$) from 41.83 (SE ±2.24) in control rats to 35.0 (SE ±1.36) and crypt-villous ratio was significantly ($P < 0.01$) increased to 18 (SE ±0.01).

## DISCUSSION

Starvation in rats was shown to be associated with a decrease in proliferating cells and prolongation of the cell renewal cycle (4). This may be due to a specific effect of lack of food or due to a general response to negative nitrogen balance produced by starvation (4). Upon refeeding, these changes reversed (4). So far, epithelial cell counts in crypts and villi of suckling Wistar rats during ST-toxin induced secretory response and during ST-toxin induced secretory response after starvation have not yet been documented. It is possible that ST-toxin which produces cGMP was acting in a manner similar to alpha-adrenergic stimulation (norepinephrine which also releases cAMP and can cause stimulation of crypt and villi proliferation by itself or through inhibition of locally active chalones which regulate cell proliferation) (5).With 24-hour fasting, the effect of fasting and that of ST-toxin complemented each other: the actively proliferating crypt cell count was increased (possibly due to stimulation by ST-toxin) whereas the villi count was reduced (possibly due to fasting).

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