EFFECTS OF INGESTED GOSSYPOL ON THE DUODENUM OF RATS

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ABSTRACT. — A significant increase in the mitotic index and rate of cell extrusion occurred in the duodenum of rats fed gossypol-containing cottonseed meal for 30 days. No histopathological effects in the mucosa, submucosa or muscularis were observed.

Limitations have been imposed upon the use of cottonseed meal as a food source because of the presence of a toxic phenolic pigment, gossypol. This substance is highly toxic to non-ruminant animals and has been shown to accumulate in the organs of some animals (Sharma et al, 1966; Smith and Clawson, 1965). The variability in the toxicity of gossypol suggests that there is likely a greater intestinal absorption of gossypol rather than an effect on variation in the metabolic reactions of the animals (Bressani et al, 1964). The manner in which gossypol is absorbed by the intestinal mucosa and transported across the intestinal wall is presently under study in our laboratory. The effect of ingested gossypol on the cell dynamics of the duodenal region of the small intestine of rats was undertaken to determine any histological changes that may influence absorption studies.

MATERIALS AND METHODS

The animals used in this study were from a colony of CF-E rats maintained in this laboratory. Twenty-four male rats (30 days of age) were randomly selected, housed individually, and allowed food and water *ad libitum*. Twelve control rats were fed a commercial rat diet in meal form and twelve experimental rats were fed the commercial diet containing 20% cottonseed meal by weight. The cottonseed meal contained 0.26% free gossypol and 0.92% total gossypol. All animals were maintained on diets for 30 days.

Rats were killed between 8:00 and 9:00 a.m. by cervical dislocation. The abdomen was opened immediately, and a section of the duodenum approximately one-half to three-fourth centimenter long was removed about five millimeters posterior to the pylorus. Pieces of the duodenum were fixed overnight in 10% neutral formalin. After fixation, the tissues were dehydrated in ethanol, cleared in xylene, and embedded in a mixture of 90% paraffin, 10% bayberry wax at 58C. Cross sections of the duodenum were cut at six microns and stained with Harris' hematoxylin and eosin.

All stages of mitosis of the epithelial cells lining the crypts were counted. Only those crypts which were sectioned throughout their entire length were used for mitotic counts. The number of interphasic

and mitotic epithelial cells in histological sections was determined by counting their nuclei. The nuclei of chief and goblet cells were included in the counts, but not those of migrating lymphocytes and Paneth cells. All counts were made with oil immersion (970X). The number of mitotic figures, interphasic nuclei, and total nuclei were recorded for 40 crypts for each animal.

In determining the percentage of villi with extrusion zones, only those villi were examined in which the section passed through the tip, as shown by the presence of a continuous single layer of nuclei in the epithelium. Forty to sixty villus tips were examined per animal and classified as to whether they did or did not have extrusion zones. The results are expressed as the per cent of villi having extrusion zones.

TABLE 1. — Mitotic Index (M.I.) of the Duodenum in Male CF-E Rats.

Diet	Number of Rats	M.I. (%)[1]
Control	12	5.41±.26
Experimental	12	7.53±.17[2]

[1] Mean ± standard error
[2] $P < 0.01$

TABLE 2. — Mean per cent duodenal villi with extrusion zones in male CF-3 rats.

Diet	Number of Rats	Villi With Extrusion Zones (%)[1]
Control	12	34.2±.32
Experimental	12	40.3±.27[2]

[1] Mean ± standard error
[2] $P < 0.01$

RESULTS AND DISCUSSION

The mitotic indices of the crypt cell population of the rat duodenum are presented in Table 1. There is a significant ($P < 0.01$) increase in the mitotic index of the duodenual crypt cell nuclei in the experimental animals. Mitosis normally occurs only in the cells lining the crypts. In the present study, mitotic figures were observed only in spaces at the base of the villi.

The percentage of villi having extrusion zones in the experimental animals is significantly ($P < 0.01$) greater than in the controls (Table 2).

It has been shown that the epithelial cells on the villus originate from proliferating cells in the crypt of Lieberkühn and then migrate up the sides of the villus to be extruded at the apex of the villus (Leblond and Messier, 1958). A delicate balance is believed to exist between the rate of cell proliferation in the crypts and the number of cells being extruded at the apex of the villus. In experiments conducted on rats, Leblond and Steven (1948) determined that 1.57 days were required for a cell to migrate from the crypt to the apex of the villus in the duodenum.

The increased mitotic index and presence of villi with extrusion zones in the duodenum of gossypol-fed rats indicate a rapid turnover of intestinal epithelium. This greater turnover of intestinal epithelium among the experimental animals suggests several possible effects of ingested gossypol. The rapid turnover may serve to renew the mucosal lining from trauma of gossypol and gossypol derivatives found in the cotton-

seed meal. Further, this increase may provide extra enzymes necessary for the hydrolysis of cottonseed meal.

Rats fed cottonseed meal did not exhibit any histopathological effects such as inflamatory reactions and necrotic erosion of the mucosa. The submucosa and muscularis appeared histologically normal among the experimental animals.

Literature Cited

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