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Impairment of salicylate uptake from rat small intestine following pretreatment with a folic acid antagonist

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Amethopterin, a folic acid antagonist, has been shown by Bognel (1965) to inhibit active transport of glucose in the intestine, and Robinson, Antonioli & Vanotti (1966) found that *in vitro* it reduced uptake of L-phenylalanine by rat intestine. Its effect on salicylate which is not absorbed from the intestine by an active process, and its uptake *in vivo* have been examined.

Male Wistar rats, 220–230 g, were treated with amethopterin (Lederle) 20 and 40 mg kg^{-1}, 48 h previously; the subcutaneous route was chosen to obviate any effects of a high local concentration in the gut. The animals were fasted overnight anaesthetized with sodium pentobarbitone, 60 mg kg^{-1} (s.c.) and the whole length of the small intestine was perfused according to Schanker, Tocco & others (1958). Sodium salicylate 50 $\mu g\ ml^{-1}$ in Krebs solution at 37° was passed through the gut at 2 ml min^{-1} by peristaltic pump and blood samples (0·2 ml) were taken at 30 min intervals and assayed fluorometrically for total unchanged salicylate (Davison, Guy & others, 1961).

Robinson & others (1966) found marked changes in the intestinal mucosa 48 h after treatment with amethopterin (40 mg kg^{-1}, oral), so representative sections were taken from gut and were stained with haematoxylin and eosin, or in some cases by the P.A.S. technique, and micrometer readings were made at four opposite points on each section to give a mean value for total depth of tissue (from villus tip to serosal surface) and for mucosal depth (from villus tip to muscularis mucosae).

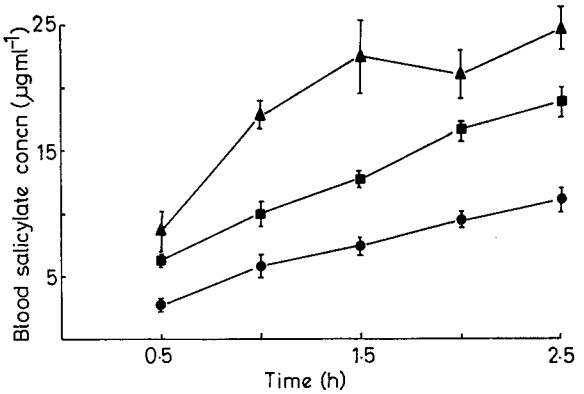

FIG. 1. Salicylate uptake from small intestine of rats pre-treated with amethopterin. ▲ Control, 0·9% saline; ■ Amethopterin 20 mg kg^{-1}; ● Amethopterin 40 mg kg^{-1}, (n = 5).

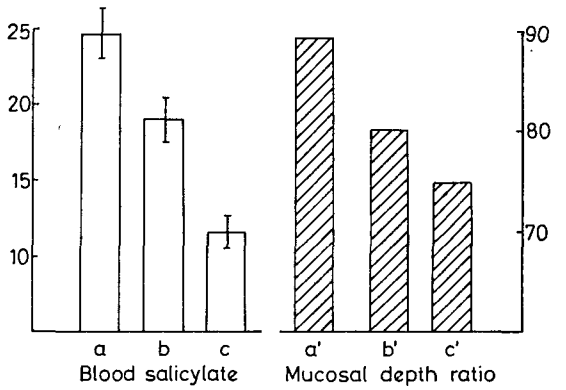

FIG. 2. Relation of mucosal changes brought about by amethopterin (MTX) to salicylate uptake from rat small intestine.

Open columns:— blood salicylate $\mu g\ ml^{-1}$, at 2·5 h (left axis) (a) saline controls; (b) MTX 20 mg kg^{-1}; (c) MTX 40 mg kg^{-1}. Hatched columns:—ratio of mucosal depth to total depth of small intestine tissue (right axis). (a') saline controls; (b') MTX 20 mg kg^{-1}; (c') MTX 40 mg kg^{-1} (confidence limits 95%).

Pretreatment with amethopterin at both doses significantly ($P = <0{\cdot}001-<0{\cdot}05$) lowered the blood salicylate (Fig. 1). This reduction appears to bear a direct relation to reduced mucosal depth found at different concentrations of amethopterin (Fig. 2).

Intestinal mucosa originates from cells in the Crypts of Lieberkuhn which move progressively towards the tip of the villus, replacing those which degenerate; the average life of intestinal epithelial cells in the albino rat being estimated at 1·5 days (Leblond & Stevens, 1948). When mitosis is prevented by the action of amethopterin there are fewer cells to replace those which are shed, so that the height and number of villi is reduced. This would diminish the surface area across which contents of the lumen could diffuse.

Amethopterin has also been reported by Vitale, Zamcheck & others (1954) to damage mucosal cells and to promote the formation of cubical or pavement cells instead of the normal columnar form. In the present study such cells were observed at doses of 20 and 40 mg kg^{-1}, but distribution was irregular and it was not possible to assess the proportion of atypical cells. Some changes in mucin distribution were seen in sections from treated animals, but could not be evaluated quantitatively.

It is not clear if the changes in cell structure or mucin distribution affected the passage of salicylate from lumen to blood, but since experimental conditions which might have affected uptake, such as pH and perfusion rate, were kept constant, it seems probable from the relation shown in Fig. 2 that the reduced area was a major factor in the decreased concentration of blood salicylate, and that the use of folic acid inhibitors may result in diminished uptake of other drugs.

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