

Communications to the Editor

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AN ASSESSMENT OF GASTROINTESTINAL MUCOSAL DAMAGE *IN VIVO*: ENHANCEMENT
OF URINARY RECOVERY AFTER ORAL ADMINISTRATION OF PHENOLSULFONPHTHALEIN
IN ULCER RATS

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The permeability of phenolsulfonphthalein(phenol red), a poorly absorbed drug, was examined as an index of an assessment of gastrointestinal mucosal damage. The urinary recovery after oral administration of phenol red was significantly increased in rats with indomethacin-induced ulcers. A similar result was obtained in rats with ulcers induced by restraint and water immersion stress. However, the urinary recoveries of phenol red after the intravenous administration in both types of ulcerated rats did not change compared with the controls. These findings suggest that the increase in urinary recovery of phenol red is due to increased gastrointestinal absorption. This method may be utilized as a simple, useful, and noninvasive screening test for an assessment of gastrointestinal mucosal damage *in vivo*.

KEYWORDS — phenolsulfonphthalein; phenol red; assessment of gastrointestinal mucosal damage; stress ulcer; restraint and water immersion stress; indomethacin; gastrointestinal absorption

For ulcer disease, numerous attempts have been made to develop disease models corresponding to the human disease in at least some respects. Gastric lesions are produced in rats by cold stress, by restraint and water immersion stress, by nonsteroid anti-inflammatory drugs including indomethacin, aspirin, and phenylbutazone, and by secretagogues. It is generally recognized that length, area, and depth are measured as a quantitative assessment of ulcer in experimental animals. The diagnosis of gastric and duodenal ulcers in humans can be made in most instances by X-ray diagnosis and endoscopic examination. However, few studies have been done to investigate the assessment of ulcer and gastrointestinal mucosal damage using the permeability of marker compounds in experimental animals. An attempt has been made to examine the intestinal mucosa in coeliac disease by permeability of cellobiose and mannitol in humans.¹⁾ We postulated that the gastrointestinal mucosa in drug-induced mucosal damage and in ulcer disease would be permeable to a poorly absorbed compound, phenolsulfonphthalein(phenol red). The present study was undertaken to investigate urinary recovery after oral administration of phenol red as an index of assessment of gastrointestinal mucosal damage *in vivo*. Phenol red was chosen as a marker compound due to its poor absorbability at any physiological pH of the gastrointestinal tract, rapid renal tubular secretion, and ease of assay.

Phenol red, indomethacin, and carboxymethyl cellulose sodium salt(CMC) were of reagent grade. All other reagents used in these experiments were of the finest grade available. Male Wistar albino rats, weighing approximately 200 g, were used in these studies.

Indomethacin-induced ulcer: After fasting, rats were administered indomethacin suspended in 1 % CMC solution by gastric intubation under light ether anesthesia and then starved, with water allowed

ad libitum. Fifteen hours later, the experiments on urinary excretion were carried out.

Restraint and water immersion stress ulcer: The method described by Takagi and Okabe was used.²⁾ Rats were immobilized in each compartment of the stress cage. The cages were then immersed in a water bath kept at 23°C for 14, 16, and 19 hours to the height of the xiphoid of the animals. After the stress load, the experiments on urinary excretion were carried out.

The rats were administered phenol red 2 μmol in 2 ml of saline by gastric intubation under light ether anesthesia. Following intubation, the animals were placed in a metabolic cage. The urine was collected for 8 hours, and phenol red content of the voided sample was determined. Spectrophotometric determination of phenol red was applied. Sample solution was alkalinized with 1N NaOH and determined

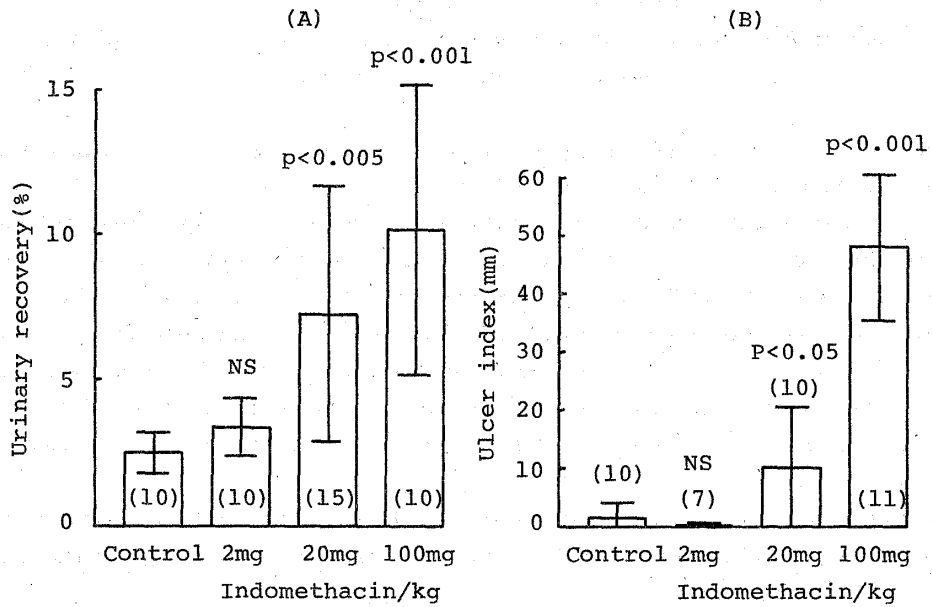


Fig. 1. Urinary Recovery of Phenol Red in 8 Hours(A) and Ulcer Index(B) in Rats Orally Pretreated with Indomethacin
Vertical bars indicate ± S.D. NS means not significantly different from the control. Numbers in parentheses represent the number of experiments.

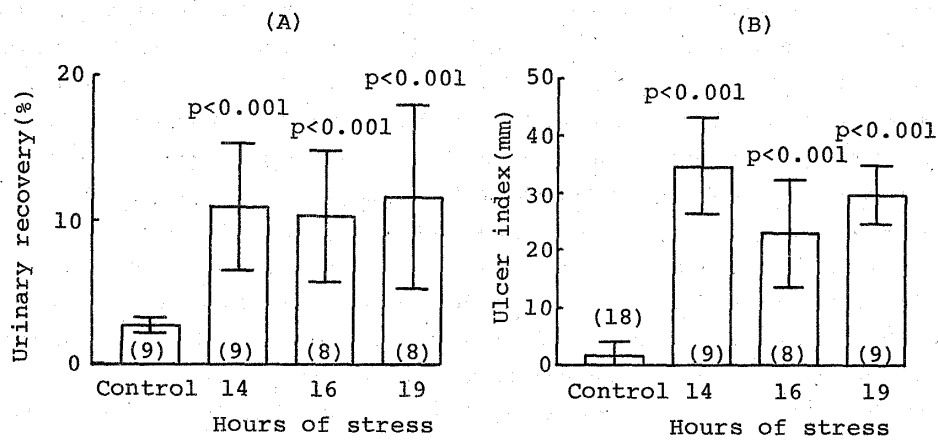


Fig. 2. Urinary Recovery of Phenol Red in 8 Hours(A) and Ulcer Index(B) in Rats Subjected to Restraint and Water Immersion Stress
Vertical bars indicate ± S.D. Numbers in parentheses represent the number of experiments.

spectrophotometrically at 560 nm. The removed stomachs were inflated with 10 ml of saline and placed in 1 % formalin solution for 5 min to fix the outlayer. The stomach was cut open along the greater curvature and the lesions in the stomach were measured. The ulcer index was expressed as the sum total of the length(in millimeters) of individual lesions on each animal. Results were compared statistically using Student's t-test.

Phenol red is almost completely ionized at pH above 1. Schanker *et al.* have shown that phenol red was poorly absorbed from the rat stomach and small intestine.^{3,4)} Also, we have shown that the poor absorbability of phenol red is due to its very low affinity to the intestinal mucosa in addition to its poor lipid solubility.^{5,6)} Phenol red given by intramuscular or intravenous injection is excreted mostly by tubular secretion and is used in the testing of renal function in humans.

The effects of oral pretreatment with indomethacin or restraint and water immersion stress on the urinary excretion after oral administration of phenol red were examined in rats. The results are shown in Fig.1 and Fig.2. The urinary recovery of phenol red and ulcer index were significantly increased by oral pretreatment with either 20 mg/kg or 100 mg/kg of indomethacin, while no effect was found in the case of 2 mg/kg of indomethacin. Similarly, a significant increase in the urinary recovery of phenol red and in the ulcer index were observed in restraint and water immersion stress rats. On the other hand, urinary recovery of the dye administered intravenously did not change significantly in rats pretreated with 100 mg/kg of indomethacin or stressed for 14 hours, compared with the controls(data not shown).

These results suggest that the increase in urinary recovery of phenol red is due to the increased gastrointestinal absorption. This method may be utilized as a simple, useful, and noninvasive screening test for an assessment of gastrointestinal mucosal damage *in vivo*.

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