A Dissimilar Effect of Folic Acid Upon Growth of the Rat Kidney and Small Bowel (34736)

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The maximum known renal growth response may be induced in laboratory animals by a single injection of folic acid (1–4). This effect may be related either to a direct pteridine-induced stimulation of DNA synthesis or to a renal injury due to precipitation of the compound in the tubules. Malt (1) states that if the mechanism is indeed one of direct pteridine-induced stimulation, the model will provide an invaluable system for studying the control of renal growth. A method for seeking information relevant to this question is to determine the effect of folic acid upon the growth of another organ, the gut.

Methods. Five female Sprague-Dawley rats, weighing 200–220 g, were given a single intraperitoneal dose of 25 mg of folic acid. Five control animals received an equal volume of intraperitoneal water. At 72 hr, the animals received 100 μCi of tritiated thymidine intraperitoneally and were deprived of solid food. The animals were sacrificed with ether after 18 hr more. The kidneys and intestines were examined; the right kidneys were blotted, weighed, and biopsied. The jejunum was biopsied 9 cm distal to the ligament of Treitz. The ileum was biopsied 9 cm proximal to the ileocecal junction. The specimens were fixed in 10% formalin, sectioned transversely to the lumen at 5 μ, and stained with hematoxylin and eosin. Radioautographs were prepared by coating slides with Kodak NTB3 emulsion, exposing for 4 weeks, developing, and counterstaining with nuclear fast red. The slides were evaluated "blind" to eliminate bias in the interpretation of the results. Measurement of the villus height and of the height to which labeled cells had migrated from the crypts was performed with an eyepiece micrometer for the five tallest well-oriented villi in each section. An index of proliferative activity for the kidney was derived as a percentage of labeled cells observed in 100 oil immersion fields for each animal. The Student's t test was employed to test the significance of differences between means.

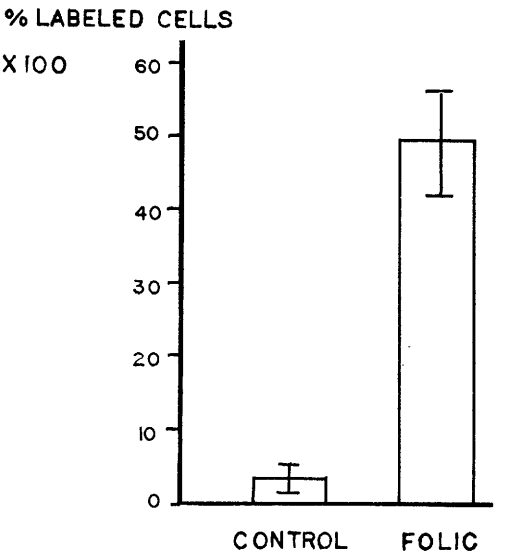

FIG. 1. Percentage of cells labeled by tritiated thymidine in kidneys of control and folic treated rats.

Results. The kidneys of the animals treated with folic acid appeared grossly enlarged and pale. The digestive tracts appeared normal in both groups. The mean weight of the right kidneys of the folic group was 0.93 g, as compared with 0.81 g in the control group. A tenfold increase in the percentage of labeled tubular epithelial cells was noted in the renal radioautographs of the folic rats ($p < .01$) (Fig. 1). In the hematoxylin and eosin sections the tubules of the

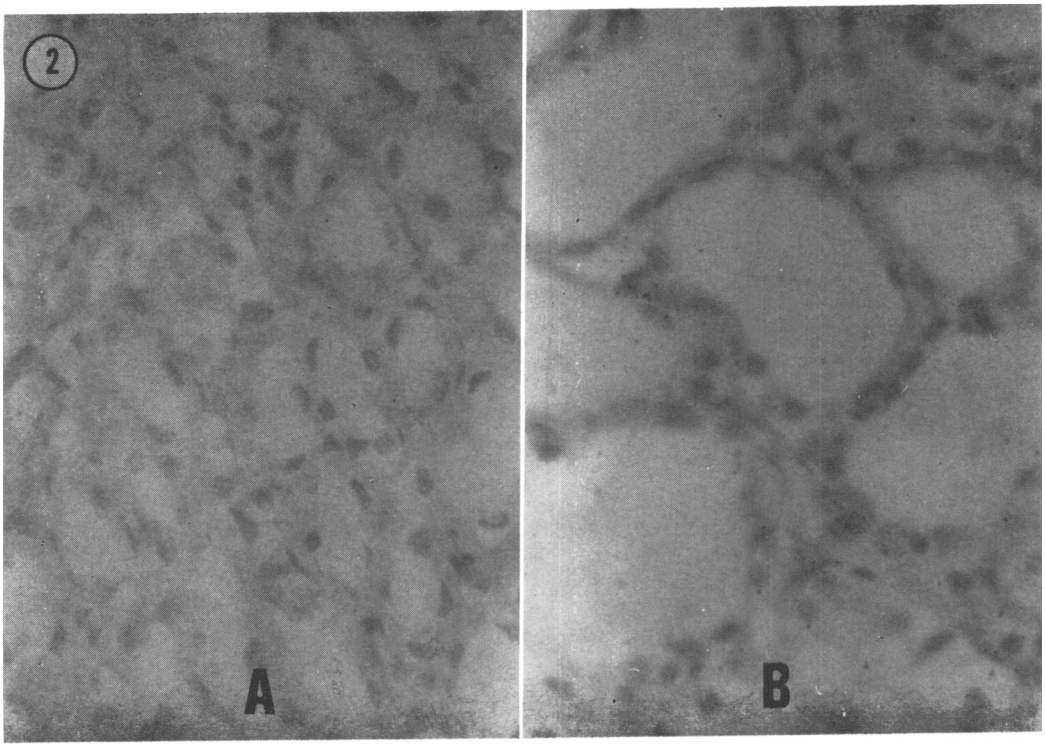

FIG. 2. Radioautographs at 400× (A) control; and (B) folic-treated kidneys showing more frequent label, more dilated tubules, and less frequent red blood cells in the experimental group.

folic treated group appeared dilated and small vessels containing red blood cells were rare (Fig. 2). The tubular and vascular architecture of the control kidneys appeared normal, with abundant red blood cells apparent in the small vessels.

There was no enhancement of villus growth after folic treatment. In fact, the jejunal villus length and the ileal cell migration rate were slightly decreased ($p < .05$ and $< .001$) in the folic treated rats (Table I). The villi appeared normal in routine sections.

Discussion. The results of this study are consistent with a body of evidence suggesting that the mechanism of renal growth stimulation by folic acid is a variation on the theme of growth after renal injury. Baserga and co-workers (2) have noted precipitates within the renal tubules; and Taylor and co-workers (3) have reproduced the growth response by the intratubular precipitation of uranyl nitrate. Additional evidence is the finding by Threlfall and co-workers (4) that the early increase in weight is due to an increase in wet weight. The dilated tubules noted in this study are consistent with the hypothesis that folic-induced hypertrophy most closely resembles the experimental model of ureteral obstruction.

The infrequency with which red blood cells were noted in the folic-treated kidneys suggests that the kidneys may have suffered an additional ischemic insult due to an increase in intracapsular pressure from the tubular obstruction. Since ischemic injury has been shown to enhance renal hypertrophy (5) and also to stimulate intestinal villus growth (6), it is possible that this factor provides a further stimulus to cell proliferation in the tubules.

Summary. The effect of folic acid upon cell proliferation in the gut and in the kidney was investigated. The growth-inducing effect of folic acid upon the kidney was confirmed, but there was no evidence of increased cell proliferation or villus hypertrophy in the gut. These findings suggest that the effect of folic acid upon renal growth is not due to a direct

TABLE I. Villus Length and Cell Migration Rates at 18 hr in Ileum and Jejunum from Control and Folic-Treated Rats.

		Ileum (μ/10 ± SE)		Jejunum (μ/10 ± SE)	
	N	Villus length	Migration rate	Villus length	Migration rate
Control	5	43.0 ± 1.6	27.2 ± 0.8	62.6 ± 2.1	29.2 ± 1.3
Folic	5	43.4 ± 1.0	23.0 ± 1.2	58.0 ± 3.1	27.4 ± 1.2
p		ns	<.001	<.05	ns

pteridine-induced stimulus but rather to a renal injury akin to ureteral obstruction.

1. Malt, R. A., N. Engl. J. Med. **280**, 1446 (1969).

2. Baserga, R., Thatcher, D., and Marzi, D., Lab. Invest. **19**, 92 (1968).

3. Taylor, D. M., Threlfall, G., and Buck, A. T., Biochem. Pharmacol. **17**, 1567 (1968).

4. Threlfall, G., Taylor, D. M., and Buck, A. T., Amer. J. Pathol. **50**, 1 (1967).

5. Lytton, B., Schwartz, S. J., Freedman, L. R., and Thompson, J. W., J. Urol. **100**, 128 (1968).

6. Tilson, M. D., Arch. Surg. (1970) in press.

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