supported by both these findings and the failure of propyl gallate to affect the hepatic uptake of the administered $^{14}CCl_4$.[17] A possible competitive effect of propyl gallate on the metabolism of carbon tetrachloride by the endoplasmic reticulum, during this short term experiment, could not be excluded, since the hexobarbital sleeping time has been found to be longer in the rats treated for 5 days with either propyl gallate or *N-N'*-diphenyl-*p*-phenylenediamine.[17] The partial failure to prevent intoxication during the later phases could be explained by comparing the actual levels of both the antioxidants and carbon tetrachloride (and/or the CCl_4-metabolites) in the liver. In fact, smaller doses of the poison (e.g. 25μl/100 g of body weight) allow propyl gallate protection better than those reported here, although the antioxidant properties found in the liver appear to be progressively exhausted at 5 up to 11 hr after dosing.[17]

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Stimulation of renal *p*-aminohippurate transport by folic acid*

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THE KIDNEY responds with a marked increase in DNA synthesis to variety of stimuli, including unilateral nephrectomy,[1] temporary ischemia,[2] metabolic acidosis,[3] mercuric chloride necrosis,[4] and folic acid administration.[5] It has been shown that a single injection of folic acid causes an increase in kidney weight and RNA synthesis within 6 hr after injection, while DNA synthesis and dry weight increase within 24 hr and reach a maximum level by 96 hr.[5–7] The increase in DNA content and the associated increase in kidney mass after administration of folic acid appear to be due to hyperplasia of the renal tubules.[5] Since folic acid appears to be a specific growth stimulant for the kidney,[5] it

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was of interest to determine if this increased growth was also accompanied by an increase in some physiological renal function. The ability of renal cortical slices to transport organic acids and bases after treatment of rats with folic acid was therefore investigated, using the method *in vitro* of Cross and Taggart.[8] The experiments described in this report indicate the effectiveness of folic acid in stimulating certain parameters of renal function.

Weanling, male, Sprague–Dawley rats were used in all experiments. The animals used were 50–60 g at the start of each experiment and were allowed free access to food and water at all times. Folic acid (Nutritional Biochemicals Corp.) was dissolved in 0·01 M sodium carbonate and immediately injected intraperitoneally (i.p.) in doses of 125 and 250 mg/kg. Control animals were given 0·01 M sodium carbonate i.p. All rats were given a single injection of folic acid or sodium carbonate and used 2, 3 or 4 days after injection.

The animals were killed by cervical dislocation and the kidneys were immediately removed, weighed and placed in ice-cold normal saline. Renal cortical slices weighing about 100 mg were prepared freehand and kept in cold normal saline until used. Slices were incubated in the phosphate buffer media devised by Cross and Taggart,[8] which contained $7{\cdot}4 \times 10^{-5}$ M *p*-aminohippurate (PAH) and $6{\cdot}0 \times 10^{-6}$ M ^{14}C-*N*-methylnicotinamide (NMN). The amount of isotope present was 0·025 μc/ml. All incubations were carried out in a Dubnoff metabolic shaker for 90 min at 25° under a gas phase of 100% oxygen. At the end of the incubation, the slices were quickly removed from the media, blotted, weighed and macerated in 3 ml of cold trichloroacetic acid (10%). A 2-ml aliquot of media was also treated with trichloroacetic acid. PAH was estimated by the method of Smith *et al.*,[9] while ^{14}C-labeled NMN was counted in a Beckman LS-100 liquid scintillation counter, using 1·0 ml of slice or media homogenate in 10 ml of modified Bray's solution (6 g of 2, 5-diphenyloxazole and 100 g of napthaline per liter of dioxane). The transport that occurred was expressed as the slice to medium (S/M) ratio (concentration of PAH or NMN/g of tissue divided by the concentration of PAH or NMN/ml of media).

Data obtained were analyzed statistically using Student's *t*-test, group comparison.[10] The 0·05 level of probability was used as the criterion of significance.

A single injection of folic acid (250 mg/kg) caused a marked increase in the kidney weight/body weight ratio within 2 days of administration (Fig. 1). This response was greatest 2 days after drug treatment

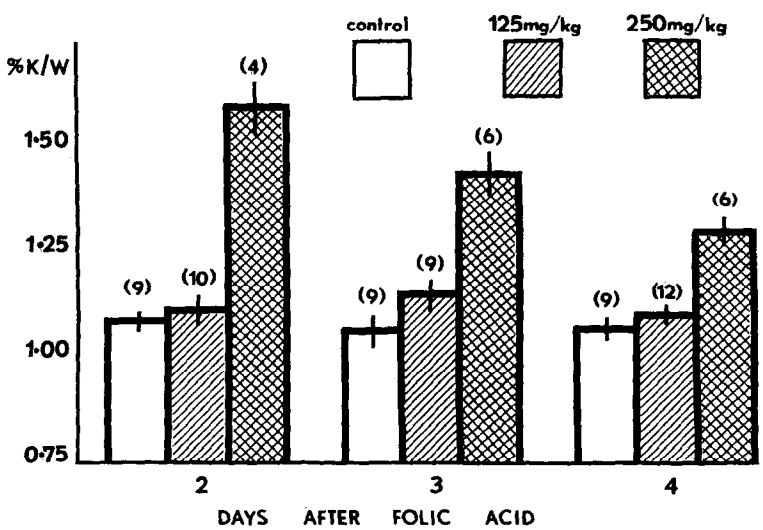

FIG. 1. Effect of folic acid administration to weanling rats on kidney weight. Results are expressed as per cent kidney weight/body weight and represent duplicate values obtained from the number of animals shown in parenthesis. Treated animals received a single injection of 125 mg/kg or 250 mg/kg of folic acid and were killed 2, 3 or 4 days later. Control animals received 0·01 M sodium carbonate. In all cases, only the effect produced by 250 mg/kg of folic acid was significantly greater than control.

and then gradually declined; however, the ratio was still significantly greater than control 4 days after folic acid injection. In contrast, 125 mg/kg folic acid did not significantly increase kidney weight.

Folic acid administration to weanling rats caused a significant stimulation of PAH transport in renal cortical slices (Fig. 2). Administration of 125 or 250 mg/kg of folic acid resulted in PAH S/M ratios *in vitro* of 9·32 and 10·81 respectively, when determined 2 days after folate injection. Both of these responses were significantly greater than the control value of 5·90. Similar results were obtained at 3 and 4 days after folic acid injection (Fig. 2).

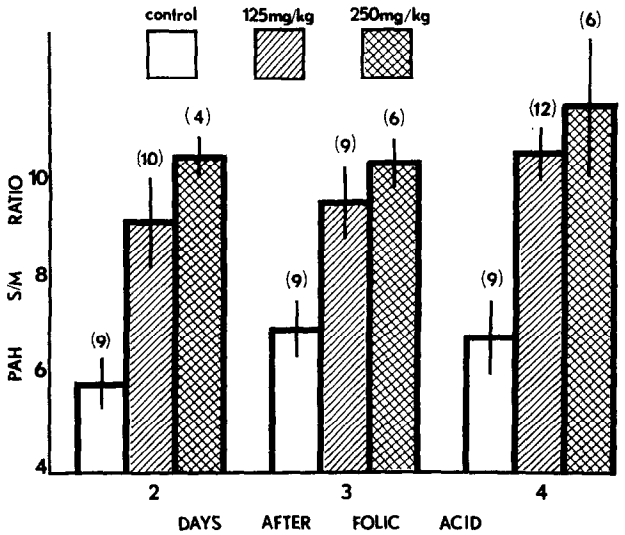

FIG. 2. Effect of folic acid administration to weanling rats on PAH accumulation in renal cortical slices. Values represent duplicate determinations from the number of animals shown in parenthesis. Treated animals received a single injection of 125 mg/kg or 250 mg/kg of folic acid and were killed 2, 3 or 4 days later. Control animals received 0·01 M sodium carbonate. In all cases both treatment effects were significantly greater than control.

Organic base (NMN) transport, on the other hand, was not stimulated by folic acid (Fig. 3). On the contrary, the high dose of folic acid led to a statistically significant reduction of NMN uptake 2 and 4 days after treatment.

In several previous reports concerning the effects of folic acid on the kidney, the folic acid was dissolved in 0·3 M sodium bicarbonate and administered intravenously.[5–7] Baserga, *et al.*[11] recently reported that intraperitoneal injection of folate dissolved in sodium carbonate gave more reproducible results. Preliminary experiments in this laboratory confirmed this latter observation, consequently this method was used in the current study. Since 0·01 M sodium carbonate was less toxic than the 0·3 M solution, this lower concentration was used.

The results presented here demonstrate that folic acid administration initiates a marked increase in the transport of PAH by renal cortical slices. The time course of this effect corresponds with the folic acid-induced increase in DNA synthesis observed by various workers including Baserga *et al.*[11] By using autoradiography, they found a marked increase in DNA synthesis in the kidney with the onset occurring at 20 hr and peaking between 38 and 60 hr after injection of folic acid. It therefore appears that this effect of folic acid coincides with its ability to stimulate the transport of organic acids in the kidney. The inability of folic acid to stimulate NMN transport indicates that the organic base transport system may be more difficult to stimulate or that folic acid is not an effective stimulus Inhibition of NMN transport could be merely an artifact in that a portion of the increase in kidney weight after folate is water.[6] Consequently, the absolute amount of transport protein per gram of slice would be reduced, resulting in an apparent decrease in transport.

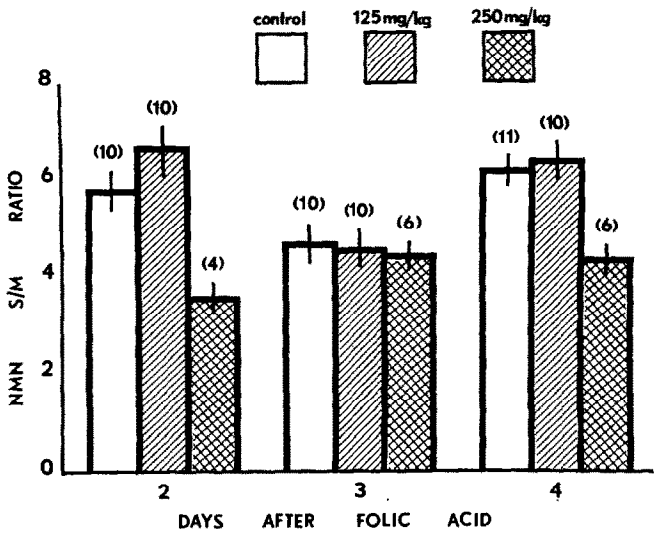

FIG. 3. Effect of folic acid administration to weanling rats on NMN accumulation in renal cortical slices. Experiments were done as described in Fig. 2. The effect of 125 mg/kg of folic acid was not significant at any time, while 250 mg/kg caused a significant depression of the NMN S/M ratio 2 and 4 days after administration.

The mechanism by which folic acid causes renal hypertrophy and hyperplasia is not completely understood. Taylor *et al.*[7] suggested that these changes are the result of partial tubular blockage caused by precipitation of folic acid within the tubules. They indicated that the effect of tubular blockage is to increase markedly the functional load on the remaining intact tubules, which initiates stimulation of RNA and DNA synthesis and ultimately causes hypertrophy and hyperplasia of the tubules. By this reasoning, synthesis of additional transport sites in these tubules would be expected to occur. The increased activity of the remaining intact tubular cells, together with the newly synthesized transport sites, would then combine to enable the kidney to transport more PAH *in vitro*. This, however, cannot entirely explain the enhanced PAH transport, for the lower dose of folic acid stimulated PAH transport without increasing kidney weight. Furthermore, NMN transport was either unchanged or depressed by folate treatment. Thus, the effect of folic acid on renal transport appears to be specific for anionic (PAH) transport and is not a generalized growth phenomenon.

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Efficacity in experimental induced liver damage of a natural polypeptide

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TWO NINHYDRINPOSITIVE fractions were obtained from a liver extract through fractionation on a cation exchanger (of SE-Sephadex C 25). Characterisation of the fractions was done by ascending chromatography on paper (*n*-propanol : NH_3: H_2O = 6:3:1). One of the fractions was pure and, after total acid hydrolysis, was shown to contain nine amino acids: Glu, Asp, Gly, Lys, Leu, Thre, Ser, Isoleu, Ala. This fraction, after experimental demonstration of its liver-protective effect, was named hepatotrop factor 1 (FH 1).

The study of the liver protective effect was carried out in two ways: (1) acute allyl alcohol intoxication in rats. In three successive experiments on 180 Wistar male rats, 180–200 g, allyl alcohol was administered per os in amount of 0·4 ml(sol 1%)/100 g animal weight. Groups of animals were preventively treated with FH 1 (solution of 8μM/ml) given subcutaneously (s.c.) at the doses of 1 ml/100 g animal weight 48·24 and 1 hr before the intoxication. Other groups were treated curatively with the same doses of FH 1, but given 1, 6, 24 and 30 hr after allyl alcohol administration. All the groups were sacrificed 48 hr after the allyl alcohol administration, the results obtained are listed in Table 1 and Fig. 1.

(2) Chronic intoxication with CCl_4 in rats. In three successive experiments the CCl_4 was administered twice weekly (six administrations) s.c. in doses of 0·1 ml/100 g to 120 Wistar male rats, of 150–200 g, divided in lots of twenty animals. FH 1 was administered s.c. at a dose of 0·5 ml/100 g animal for 5 days after the end of CCl_4 administration. Twenty-four hr after the end of the FH 1

TABLE 1. THE EFFECT OF FH 1 ON THE LIVER DAMAGE INDUCED WITH ALLYL ALCOHOL IN RATS IN g OF DAMAGED LIVER TISSUE FOUND FOR A GROUP OF TWENTY RATS

No. of experiments	Denomination of group treated		
	control	preventively	curatively
I	8·328	0·720	1·300
II	7·070	0·608	1·560
III	7·955	0·570	1·648

FH 1 was administered preventively for an accumulation in liver and curatively (1–30 hr after intoxication) of the reason then the liver necrosis become clearly visible in allyl alcohol intoxication after 36 hr. Of every liver lobe the damaged tissues was weighed. In this way a total value of damaged tissues is found for twenty animals group which can be compared with that of a control group (allyl alcohol intoxicated).