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Correspondence re: S. E. Dew *et al.*, Effects of Pharmacological Retinoids on Several Vitamin A-metabolizing Enzymes. Cancer Res., *53:* 2965–2969, 1993.[1]

Currently there is much interest in the anticarcinogenic potential of the synthetic retinoid, 4-HPR[2] and in the mechanisms by which it causes decreases in plasma levels of retinol and RBP. In a recent article, Dew *et al.* (1) described the effects of 4-HPR on the activities of several vitamin A-metabolizing enzymes. They reported that 4-HPR inhibits the activities of intestinal lecithin:retinol acyltransferase and retinal reductase. On the basis of their results, the authors speculated that the ability of 4-HPR to "inhibit retinal reduction and retinol esterification *in vitro* suggests an ability to interfere with normal vitamin A metabolism *in vivo*, particularly during absorption."

Of interest in light of this hypothesis, we recently measured the effects of 4-HPR on vitamin A absorption in the lymph duct-cannulated rat. Although Smith *et al.* (2) have shown that i.v. administration of the drug to rats causes a rapid decrease in plasma levels of retinol and RBP, we reasoned that a possible effect on vitamin A absorption might also contribute to the chronic consequences of the drug. Eleven male Sprague-Dawley rats (270–443 g) were prepared for lymph collection as described previously (3, 4); all procedures were approved by Penn State University's Animal Care and Use Committee. During a 2-day recovery period, rats were offered a fat-free diet and an electrolyte drink, sterile saline (2.4 ml/h) and corn oil (0.062 ml/h) containing 42 nmol of retinyl palmitate/g (equivalent to the presurgical fat and vitamin A intake) were infused into the duodenum through a double lumen catheter. In previous studies, we have shown that, under this setup, rats digest and absorb fat normally (based on lymphatic appearance of triglycerides) during 4–7-day experiments. For the present studies, vitamin A absorption in the absence or presence of 4-HPR was measured on 2 consecutive nights after a 2-day postsurgical recovery period. For all rats, we determined vitamin A absorption in the control state before administration of 4-HPR since it was not known if there might be any residual effects of the drug on normal vitamin A absorption. At 8 p.m. on the first experimental night, rats received a 1-h equivalent of the retinyl palmitate-containing oil to which a pulse dose of [^{3}H]retinyl acetate had been added. Lymph samples of 1-h duration were automatically collected at 5°C for 19 h. Then, at 8 p.m. on the next night, the oil infusate was changed to include 4-HPR (~50 μmol/kg/day). Twenty-four h later, a second pulse dose of [^{3}H]retinyl acetate was administered and lymph samples were collected for 19 h as on the previous night. For each collection period, aliquots of pooled lymph samples were analyzed for vitamin A mass (5) and radioactivity. Because the catheters of several rats failed during the 4-HPR period, fewer values (from 6–8 rats) were obtained for that treatment.

During the control period, vitamin A absorption calculated by mass balance averaged 77 ± 6% (SEM) and was 76 ± 5% based on radioactivity. Infusion of 4-HPR did not have a significant effect on vitamin A absorption when calculated by mass balance (67 ± 12%) or [^{3}H]retinol (77 ± 10%). In both periods, essentially all of the absorbed radioactivity was recovered within 8 h after dose administration. The finding that only 5.5 ± 1.5% of the total infused tritium (control plus 4-HPR period) was recovered in carcass minus intestines indicates that absorbed retinol was quantitatively recovered in lymph.

Thus, in addition to the effects of 4-HPR on activities of intestinal vitamin A-metabolizing enzymes *in vitro*, our results indicate that, at a dose comparable to that used in human studies, acute administration of the drug to rats does not interfere with the extent of vitamin A absorption *in vivo*. Since we measured the cumulative absorption of vitamin A in the 8 h after administration of [^{3}H]retinyl acetate, we cannot rule out the possibility that a 4-HPR-related inhibition of intestinal retinol esterification via lecithin:retinol acyltransferase decreases the rate but not the extent of absorption. Based on the work of Smith *et al.* (2), it seems likely, as discussed by Dew *et al.* (1), that the primary mechanism by which 4-HPR affects plasma levels of retinol and RBP is via an inhibition of the secretion of the retinol:RBP complex into plasma from both liver and extrahepatic tissues.

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[2] The abbreviations used are: 4-HPR, *N*-(4-hydroxyphenyl)retinamide (fenretinide); RBP, retinol-binding protein.

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Reply

We agree with Allen *et al.* (1) that the principle effect of 4-HPR[1] in vitamin A metabolism is likely due to inhibition of secretion of

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[1] The abbreviation used is: 4HPR, *N*-(4-hydroxyphenyl)retinamide (fenretinide).