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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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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).

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

retinol: retinol-binding protein. However, possible inhibition of retinol uptake in the intestine perhaps cannot yet be dismissed as a contributing factor in depletion of circulating vitamin A by 4-HPR.

One important difference between the study presented and studies with actual patients is assessment of long-term effects. In clinical trials, the signs of retinol deficiency, such as night blindness and various dermatological conditions, do not immediately manifest themselves but develop in patients after some weeks or even months on the drug (2, 3). Allen *et al.* provided their rats with a single dose of 4-HPR over a relatively short period of time. As they point out, it is possible that partial inhibition of the rate of intestinal absorption did occur but could not be detected, since they measured total absorption over an 8-h period. Since the normal capacity of the intestine to process retinol exceeds the daily vitamin A requirement, partial inhibition might not greatly affect the total absorption accomplished during an acute administration of 4-HPR. However, it is possible that chronic administration might produce sufficiently high levels of 4-HPR in the intestine to affect vitamin A absorption even though acute administration does not. This possibility could be tested nicely in their system.

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