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# Absorption and Penetration of Dinoprost (Prostaglandin $F_{2\alpha}$) and Dinoprost Methyl Ester into Perfused Mesenteric Circulation in Rats


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Received March 6, 1978, from *Experimental Biology, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication February 6, 1979.



**Abstract** □ The absorption of dinoprost (prostaglandin $F_{2\alpha}$) and its methyl ester in rat jejunum was studied. A 22-cm segment of rat jejunum was cannulated at both ends and connected to an oscillating perfusion pump system. The mesenteric vasculature supplying this isolated segment also was cannulated and perfused with Kreb's bicarbonate buffer with dextran. Solutions of $^3$H-dinoprost or its methyl ester were introduced into the lumen and oscillated through the segment. The disappearance of radioactivity from the lumen and the appearance of radioactivity in the vascular perfusate were monitored. The metabolite patterns in the vascular perfusate were analyzed by TLC. A lag between the time the drug disappeared from the lumen and the time it appeared in the mesenteric circulation was detected. This lag was longer for the methyl ester than for the free acid, even though the ester disappears from the lumen faster than does the free acid. Upon removal of dinoprost from the gut lumen, a gradual decrease in the amount of drug appearing in the mesenteric circulation could be detected. However, with the ester, a slight increase could be observed for ~0.5 hr, followed by a decrease. Metabolism by the gut wall appears to be greater for the ester than for the acid. The results suggest that, although the ester disappears from the lumen more quickly than does the acid, it actually penetrates to the blood at a slower rate and undergoes greater metabolism.



**Keyphrases** □ Dinoprost—absorption and penetration, mesenteric circulation, rats, metabolism □ Dinoprost—methyl ester, absorption and penetration, mesenteric circulation, rats, metabolism □ Pharmacokinetics—dinoprost and dinoprost methyl ester, mesenteric circulation, rats □ Prostaglandins—dinoprost, dinoprost methyl ester, absorption and penetration, mesenteric circulation, rats, metabolism


Prostaglandins have been viewed as potential panaceas almost since the moment of their discovery. Much research has been devoted to their endogenous and exogenous functions in various organs and tissues, and various roles have been assigned to this family of compounds. Because of their ubiquity and functional diversity, prostaglandins are appealing prospects as therapeutic agents for many conditions. However, before this potential can be realized, a practical administration route must be developed. The most desirable route would be oral. When dinoprost (prostaglandin $F_{2\alpha}$) or its methyl ester are administered orally, the major absorption site appears to be the small intestine since there is little absorption from the stomach[1]; although there can be some absorption by the large intestine, most of the compound disappears before reaching this area (1).

The present study was designed to gain insight into intestinal absorption of prostaglandins: their disappearance from the intestinal lumen, their appearance in the mesentric circulation, and the extent of their metabolism in the intestinal wall. Investigations into the rates of prostaglandin disappearance from the intestinal lumen (1, 2) have been based on studies of prostaglandin disappearance from the mucosal side of the intestine. From such studies, Ho and coworkers (3, 4) proposed that dinoprost absorption is diffusion dependent and that its metabolism may affect the absorption rate. In addition, previous work[2] showed that the lymphatic system does not play a role in the transport of orally administered prostaglandins.

This study looked at the serosal side of the intestine *via*

[1] E. Daniels and R. VanEyk, The Upjohn Co., Kalamazoo, MI 49001, personal communication.

[2] L. Compton and J. Weeks, The Upjohn Co., Kalamazoo, MI 49001, personal communication.


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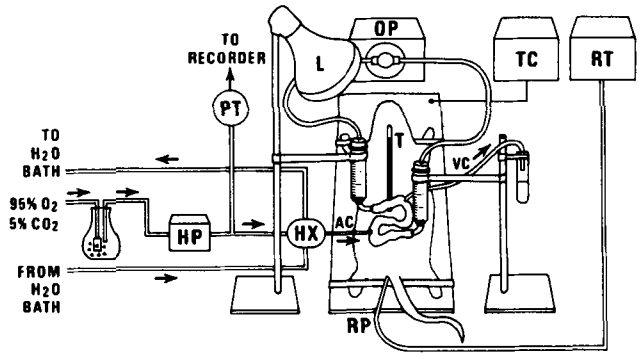


**Figure 1**—*Diagram of experimental setup. Key: HP, parastaltic pump; PT, pressure transducer; L, warming lamp; HX, heat exchanger; AC and VC, arterial cannula and venous cannula; OP, oscillating pump; TC, platform temperature controller; RP, rectal probe; RT, radio thermometer; and T, thermometer.*

the mesenteric circulation to obtain a more complete intestinal absorption profile. Since it has been proposed that prostaglandin esters are absorbed faster and achieve greater circulating levels than the free acids (2, 5), the differences in absorption between dinoprost and its methyl ester were examined.

## EXPERIMENTAL

**Materials**[3]—[9(*N*)-$^3$H]-Dinoprost was commercially prepared[4]. $^3$H-Dinoprost methyl ester was produced by reacting $^3$H-dinoprost with diazomethane.

**Methods**—Several techniques have been developed for studying intestinal absorption and transfer into the mesenteric circulation (6–10). The experimental technique employed for this study was an expansion of Ho's modified Doluisio absorption method (5) (Fig. 1).

Male Sprague–Dawley rats, 600–800 g (these larger animals were chosen because the mesenteric blood vessels were much easier to cannulate), were anesthetized with cyclopal sodium and placed on a temperature control platform[5] to maintain body temperature at 37°; body temperature was monitored with a rectal probe. The abdomen was opened, and branches of the superior mesenteric artery and vein supplying the superior jejunum were cannulated with polyethylene tubing[6].

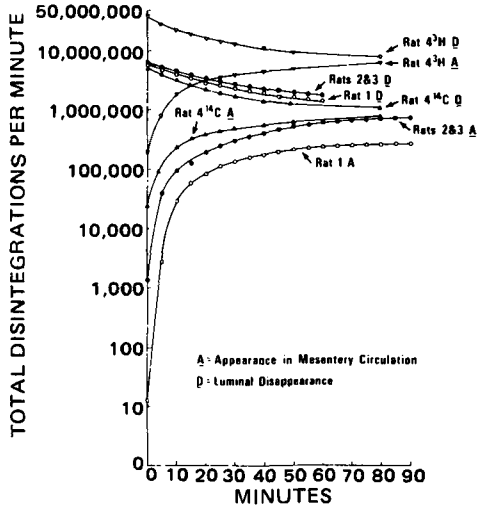


**Figure 2**—*Dinoprost disappearance and appearance curves.*

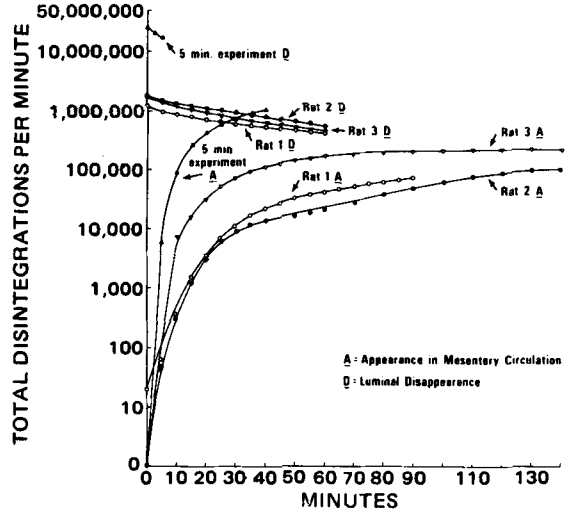


**Figure 3**—*Dinoprost methyl ester disappearance and appearance curves.*

The vasculature was perfused with an oxygenated solution of Kreb's bicarbonate buffer (pH 7.4) with 10% dextran and 150 IU of heparin/ml *via* a parastaltic pump[7]. Perfusion pressure was monitored by a pressure transducer[8]. Perfusate passed from the arterial cannula[6] through the vasculature and into the venous cannula[6], where it drained into collecting tubes. Next, a 22-cm section of jejunum supplied by the perfused vasculature was laid out in a S pattern and washed free of fecal matter. Cannulas were inserted at each end such that syringe barrels could be inserted into the cannulas and secured.

The exposed gut temperature was monitored (thermometer) and regulated with a lamp. Radiolabeled drug solution[9] (2 ml) containing 10 μg of prostaglandin in pH 6 buffer was placed in the gut, and a reversible pump[10] was connected to the syringe barrels, allowing the solution to be oscillated slowly through the gut (0.75 ml/min). [Moving the solution is reported to reduce the thickness of the aqueous diffusion layer (4).] The drug solution was prepared in isoosmotic pH 6.0 phosphate buffer (prepared by diluting 9.07 g of anhydrous monobasic potassium phosphate and 11.93 g of potassium sulfate to 1 liter with water). The pH was adjusted with 1 *N* NaOH (11). This buffer produces no water flux into or out of the gut in experiments with $^{14}$C-polyethylene glycol.

Two minutes was allowed for mixing before the first luminal fluid sample was taken, followed by sampling at 5-min intervals. Effluent from

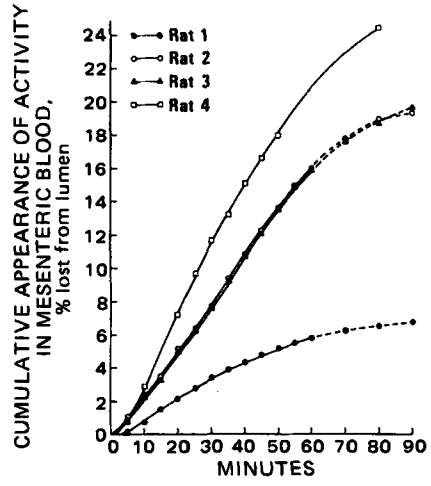


**Figure 4**—*Dinoprost appearance in mesenteric circulation. Dotted lines indicate time after drug removal from lumen.*

[3] Metabolite standards were provided by Frank Lincoln, Experimental Chemistry, The Upjohn Co., Kalamazoo, MI 49001.
[4] New England Nuclear, Boston, MA 02118.
[5] Narco Biosystems, Houston, Tex.
[6] PE-50 Intramedic, Clay Adams, Division of Becton, Dickinson and Co., Parsippany, NJ 07054.
[7] Model 911 Holter Roller Pump, Extracorporal Medical Specialities, King of Prussia, PA 19406.
[8] Harvard Apparatus Co., Millis, MA 02054.
[9] Two milliliters of drug solution had the same disappearance half-life in these large rats as was demonstrated in the smaller rats used by Ho (5).
[10] FMI lab pump, Fluid Metering Inc., Oyster Bay, NY 11771.

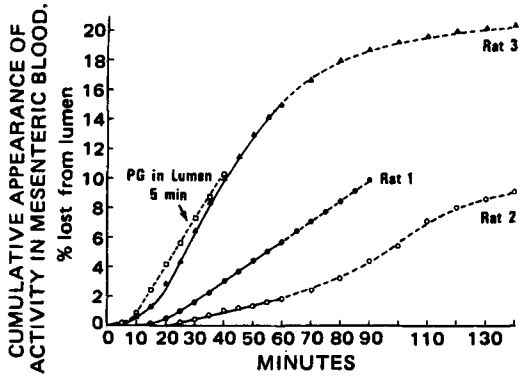


**Figure 5**—*Dinoprost methyl ester appearance in mesenteric circulation. Dotted lines indicate time after drug removal from lumen.*

the venous cannula was collected in separate tubes for each sampling interval. The drug solution remained in the gut for the designated time—usually 60 min—and was then removed; the gut was then washed free of drug. The vascular perfusion was continued for an additional period to check for drug holdup in the intestinal wall and to follow drug release. Upon completion of the perfusion, the experimental intestine section was removed, and the amount of radioactivity remaining in the wall was determined.

**Analysis**—The radioactivity in the luminal samples and that in the vascular perfusate were determined by direct counting of small aliquots in a scintillation counter[11]. These and all other counting data were corrected for quench. Intestinal wall tissue was homogenized[12] and oxidized with an oxidizer[11], and the oxidation products were counted to determine the residual radioactivity in the gut wall.

The metabolism data were obtained by pooling the vascular perfusate samples into three portions (0–30 min, 35–60 min, and 65 min–end), extracting with ethyl acetate, and subjecting the extract to TLC. TLC was carried out on silica gel plates[13] in chloroform–methanol–water (40:10:1) and then chloroform–methanol–acetic acid (40:2.5:5). The plates were scraped, and the areas where the various standards ran were noted and counted.

## RESULTS AND DISCUSSION

Several intestinal absorption parameters were determined from the data collected: (*a*) the amount of drug lost from the lumen, (*b*) the amount and pattern of drug appearance in the mesenteric circulation, (*c*) the amount of drug held up in the gut wall, and (*d*) the extent and pattern of intestinal metabolism. In addition, the esterase activity in the gut lumen was determined for the experiments with dinoprost methyl ester[14].

Figure 2 shows dinoprost disappearance from the intestinal lumen and the appearance of the label in the mesenteric circulation plotted on a log scale of the total disintegratious per minute *versus* time. By the time that the zero sample was taken (after a 2-min delay), activity had already begun to appear in the circulation and continued to increase until the drug was removed from the gut. After removal of the drug solution from the lumen, the rate of increase of radioactivity appearing in the mesenteric circulation slowly leveled off; after 30 min, the slope approached zero.

Figure 3 shows the disappearance and appearance of dinoprost methyl ester. Ester disappearance studies are complicated by the presence of a luminal esterase, which converts the ester to the free acid at a highly variable rate. TLC of the luminal extract demonstrated that the only radioactive species present were dinoprost and dinoprost methyl ester and, therefore, indicated no back-diffusion of prostaglandin metabolites into the lumen.

Some differences between the free acid and the ester should be noted. First, there is a delay between the time the drug disappears from the lumen and the time it appears in the circulation since most plots start from zero. Second, radioactivity continues to increase in the circulation

[11] Packard Tri-Carb, Packard Instrument Co., Downers Grove, IL 60515.
[12] Polytron homogenizer, Brinkmann Instruments, Westbury, NY 11590.
[13] Analtech.
[14] G. Tarpley and F. Sun, The Upjohn Co., Kalamazoo, MI 49001, 1976, unpublished data.

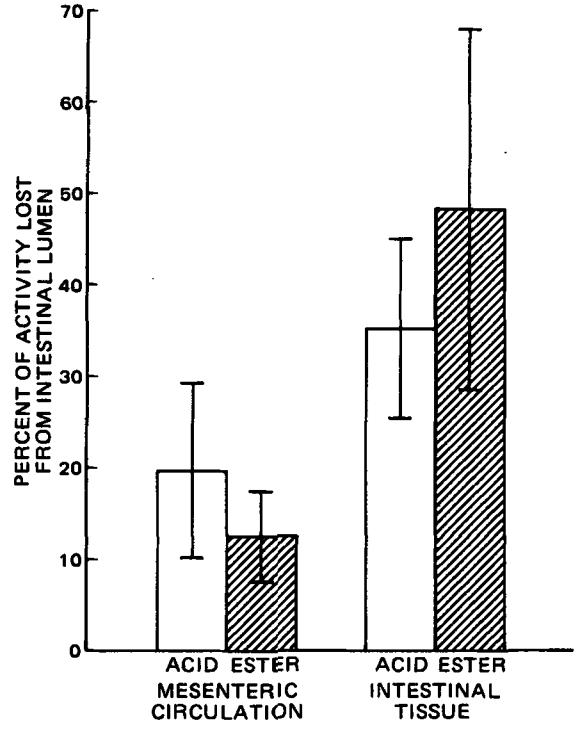


**Figure 6**—*Dinoprost and dinoprost methyl ester activity distribution. Vertical line indicates standard deviation.*

after the drug is removed from the lumen; *i.e.*, the slope remains positive. The slope of the appearance curve for Rat 3 more closely resembles the slopes for dinoprost because the esterases, as measured by the ester to acid ratio in the luminal fluid, were not as active in Rats 1 and 2 as in Rat 3.

The percent of the total activity lost from the lumen appearing in the mesenteric circulation for dinoprost *versus* time is shown in Fig. 4. The broken line indicates the time after drug was removed from the lumen. The radioactivity appeared immediately in the mesenteric circulation, and the slope began to level off shortly after drug removal. A similar plot for dinoprost methyl ester is shown in Fig. 5. There was an apparent delay before radioactivity began to appear, and the slope continued to rise even after drug removal, which was especially apparent when the drug solution was removed after only 5 min in the lumen.

Figure 6 shows the activity distribution in the two compartments, tissue and blood (plotted mean ± *SD*). These results, along with those showing

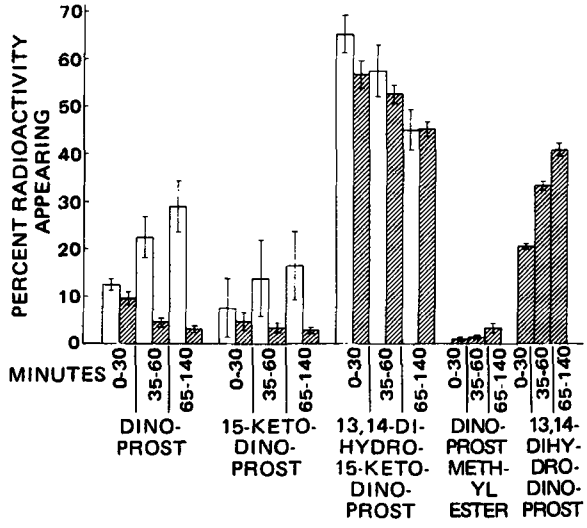


**Figure 7**—*Metabolites appearing in mesenteric circulation. Key:* □, *dinoprost in intestinal lumen; and* □, *dinoprost methyl ester in intestinal lumen.*

a delay between disappearance from the lumen and appearance in the blood, are in agreement with previous studies (12), which indicate that the greater a compound's lipophilicity, the longer will be its holdup in the intestinal wall and the less will penetrate to the serosal side of the membrane.

Finally, as is expected with longer holdup, the ester is more extensively metabolized than is the acid, as indicated by TLC. With dinoprost, the main metabolites are 15-ketodinoprost and 13,14-dihydro-15-ketodinoprost. As the experiment progressed, the metabolism decreased (Fig. 7). With the ester, four main metabolites are seen: dinoprost, 15-ketodinoprost, 13,14-dihydro-15-ketodinoprost, and 13,14-dihydrodinoprost. Although the amount of intact ester entering the blood increased slightly as the experiment progressed, the total metabolism increased, as indicated by the increase in 13,14-dihydrodinoprost in the circulation.

In summary, intestinal prostaglandin absorbed appears to have at least three phases. First, prostaglandins appear to diffuse rapidly from the gut lumen into the intestinal wall. Second, once in the gut wall, time is required for the compound to reach the other side of the cell. During this transcellular movement, the compound appears to be extensively, although not completely, metabolized. The duration of this second phase appears to be directly related to lipophilicity. Third, the compound and its metabolites are released into the mesenteric circulation. Prostaglandin esters apparently are released slower and over a longer period than the parent acids; however, they also undergo more extensive metabolism.

This study demonstrated differences in dinoprost and dinoprost methyl ester absorption patterns in the rat. However, these differences were not in agreement with the hypothesis that prostaglandin esters are absorbed faster than the parent prostaglandins in this species.

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# Conjugated Estrogens Bioinequivalence: Comparison of Four Products in Postmenopausal Women


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Received November 6, 1978, from the **Pharmacy Research and Development Department and the* ‡*Analytical Research and Development Department, Ayerst Laboratories Inc., Rouses Point, NY 12979.* Accepted for publication February 7, 1979.



**Abstract** □ The bioequivalence of four conjugated estrogens tablets USP was compared by measurement of seven estrogens or estrogen metabolites in the urine during steady-state dosing in postmenopausal women. Two studies compared three generic products with the innovator's product. The urinary excretion of 17$\alpha$-dihydroequilin, 17$\alpha$-dihydroequilenin, and 17$\alpha$-estradiol were significantly greater in all cases with the innovator's product than with the generic products. Statistically significant differences between products were observed occasionally for other components. The generic products thus were bioinequivalent to the innovator's product, although all products essentially met current compendial specifications. A third study observed no significant differences between three batches of the innovator's product for the seven components. Total conjugated estrogens excretion of all products at the steady state was essentially equal and correlated with neither disintegration time nor dissolution half-time. Bioinequivalence between products is discussed in relation to the need for an improved USP conjugated estrogens monograph. Evidence suggesting the metabolism of a fraction of dosed estrone, equilin, and 17$\alpha$-dihydroequilin to 17$\beta$-estradiol, 17$\beta$-dihydroequilin, and 17$\alpha$-dihydroequilenin, respectively, is presented.



**Keyphrases** □ Estrogens, conjugated—bioinequivalence of generic and proprietary products, postmenopausal women □ Bioequivalence—conjugated estrogens, bioinequivalence of generic and proprietary products, postmenopausal women □ Product substitution—estrogens, conjugated, bioinequivalence of generic and proprietary products, postmenopausal women


Conjugated estrogens of natural origin are composed of at least nine different estrogens or estrogen metabolites, each present in a different amount. The USP monograph for conjugated estrogens (1) contains specifications for total conjugated estrogens and for the two most abundant components, sodium estrone sulfate and sodium equilin sulfate. The sodium estrone sulfate specification is 50–65% and the sodium equilin sulfate specification is 20–35% of the total conjugated estrogens content. The monograph does not contain quantitative specifications for additional components, although an identification test (2) requires the presence of a prominent GLC peak for 17$\alpha$-dihydroequilin and additional peaks for 17$\alpha$-estradiol, 17$\beta$-dihydroequilin, equilenin, 17$\beta$-estradiol, and 9-dehydroestrone.

Broad content ranges are specified for sodium estrone sulfate and sodium equilin sulfate. The content of the third major component, sodium 17$\alpha$-dihydroequilin sulfate, which by GLC is 15% of the total estrogens in the innovator's product, is unspecified; the specification for this component is an imprecise and nonquantitative identification test. A similar requirement is made for minor components.

Pharmaceutical equivalents are defined (3) as "drug products that contain identical amounts of the identical active drug ingredient. . . ." Drug product compliance with compendial standards is generally assumed to assure pharmaceutical equivalence (4). A consequence of the broad or nonquantitative specifications for both major and minor components is that compendial standards may not




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