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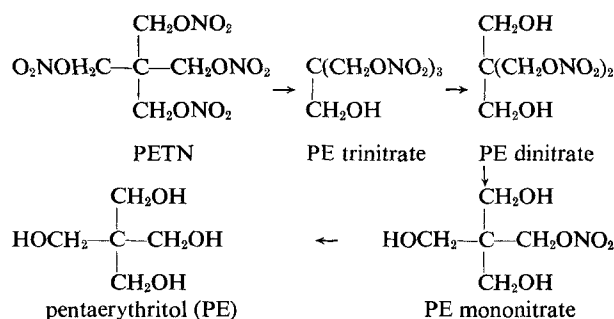
Pharmacodynamics and Biotransformation of Pentaerythritol Tetranitrate in Man

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Abstract □ The absorption, biotransformation, and excretion of pentaerythritol tetranitrate was studied after oral administration of two dosages, 20 and 40 mg., to patients. The drug was given as ¹⁴C-pentaerythritol tetranitrate incorporated into tablets of a type used clinically. The total ¹⁴C excretion in 48 hr. was approximately 92% of both doses. However, a greater proportion of the lower dose was excreted in the urine: 60% of the 20-mg. dose and 50% of the 40-mg. dose. Drug radioactivity was detected in the blood within 15 min., and peak levels occurred from 4 to 8 hr. after administration. The only radioactive compounds found in the blood were pentaerythritol, pentaerythritol mononitrate, and pentaerythritol dinitrate. These drug metabolites were also present in the urine and feces. The kinetics of renal excretion of the principal urinary metabolites, pentaerythritol and pentaerythritol mononitrate, were first order. The renal elimination-rate constant, *k_e*, of pentaerythritol was independent of the dose, but *k_e* for pentaerythritol mononitrate was dose related and significantly smaller for the higher dose. The ratio of pentaerythritol mononitrate/pentaerythritol excreted in the urine was approximately 1:1 for the lower dose and 3:1 for the higher dose. The findings indicate a rapid deesterification of pentaerythritol tetranitrate by the human to pentaerythritol mononitrate after oral ingestion, but a limited capacity for the conversion of pentaerythritol mononitrate to pentaerythritol.

Keyphrases □ Pentaerythritol tetranitrate and ¹⁴C-substituted—human pharmacodynamics, biotransformation □ Biotransformation, pharmacodynamics—pentaerythritol tetranitrate □ Urinary, fecal excretion—pentaerythritol tetranitrate □ TLC—separation □ Scintillometry—analysis

It has been generally assumed that all organic nitrates exert qualitatively similar actions and that the extended duration of action ascribed to the "long-acting" nitrates relates either to differences of absorption and metabolic stability or to specific properties of the drug molecule itself (1). Since little specific information is available on the pharmacodynamics and biotransformation of this group of drugs in man, a study was performed with pentaerythritol tetranitrate (PETN), a "long-acting" organic nitrate in wide clinical use. Biotransformation of PETN (Scheme I) was followed qualitatively and quantitatively by modifying procedures developed earlier using ¹⁴C-labeled drug (2). Drug pharmacodynamics were examined at two dose levels, 20 and 40 mg., with a clinical dosage form prepared from ¹⁴C-PETN.



Scheme I

EXPERIMENTAL

Subjects—The subjects were 15 male volunteers between the ages of 30 and 68 years who presented no history or evidence of malabsorption, intestinal motility disturbances, or renal disease. For the period of study (4 days), the subjects were restricted to the Clinical Research Unit at Bowman Gray School of Medicine. A complete medical history and physical examination were taken on each subject. Prestudy laboratory data included the serum levels of electrolytes (Na⁺, K⁺, Cl⁻, and CO₂), urea nitrogen, uric acid, blood sugar, cholesterol, inorganic phosphate, lactate dehydrogenase, total protein, calcium, bilirubin, alkaline phosphatase, and glutamic-oxaloacetate transaminase. Additional laboratory tests performed were EKG, chest X-ray, sedimentation rate, hematocrit, CBC, and urinalysis.

Drug Administration—¹⁴C-Labeled and nonradioactive PETN were used to prepare compressed tablets, which contained a total of 20 mg. of PETN and 44 μc. each and met the chemical assay and disintegration-time specifications for the manufacture of a commercial product.¹ After an overnight fast, one tablet was administered *per os* to each of 10 subjects and two tablets were administered similarly to each of five subjects. All subjects remained in the fasting state for an additional 2 hr.

Collection of Specimens—Urine was voided directly into plastic bottles stored in a dry-ice chest. The collection periods were 0–2, 2–4, 4–8, 8–12, 12–24, and 24–48 hr. after drug administration.

Immediately after defecation into a plastic container, each stool collection was covered with cold dioxane and stored in a dry-ice chest. For each subject the feces were pooled from 0–24, 24–48, and 48–72 hr.

Blood specimens (10 ml.) were withdrawn into 15-ml. EDTA-Vacutainers² at the following intervals postadministration: 15 and

¹ Peritrate.

² Becton, Dickinson Co.

30 min., 1, 2, 4, 8, 12, 24, and 48 hr. Each blood sample was transferred immediately into a labeled polycarbonate tube and frozen in a dry-ice bath.

Assays for Pentaerythritol (PE) and Its Nitrates—The total radioactivity of blood, urine, and fecal matter from ^{14}C -PETN and its metabolites was measured by liquid scintillation counting with a Packard spectrometer at 7°. Channel ratio and internal standardization methods were used to correct for quenching. The scintillation solution consisted of 8.0 g. of 2-(4-*tert*-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole (butyl-PBD) and 0.5 g. of 2-(4'-biphenyl)-6-phenylbenzoxazole (PBBO) in 1 l. of toluene to which was added 200 ml. of a solubilizer.³

PETN and its metabolites (PE trinitrate, PE dinitrate, PE mononitrate, and PE) were identified in the urine and fecal matter by extraction of total ^{14}C -radioactivity, partial purification, and then separation by TLC using 5×20 -cm. precoated (250 μ) silica gel G plates.⁴ The K solvent system consisted of toluene-ethyl acetate-butanol-water (5:5:1:1, upper phase). The R_f values, with a front movement of 10 cm., were: PE, 0.00; PE mononitrate, 0.16–0.23; PE dinitrate, 0.45–0.55; PE trinitrate, 0.60–0.70; and PETN, 0.82–0.90. The relative amounts of these compounds were estimated by radioscaning with a model 1036 Nuclear-Chicago Actigraph II radioscaner. The areas under the peaks were measured with a compensating polar planimeter, and the relative quantities of each compound were calculated from proportional areas.

The urine from each collection period for a subject was thawed and mixed and the volume was measured. Duplicate 0.5-ml. aliquots were counted for radioactivity by mixing with 15 ml. of the toluene-scintillation cocktail. For chromatography, 5 ml. of urine was evaporated to dryness with an air stream at room temperature. The residue was extracted with 1.0 ml. of methanol-ethyl acetate (1:1, v/v), clarified by centrifugation, and concentrated by evaporation with a nitrogen stream to a minimum volume for spotting on a TLC plate.

The feces of a subject for each collection period were thawed and thoroughly mixed in its container with a Servall Omni-mixer. The feces-dioxane slurry was filtered with vacuum, and the residue was reextracted three times with 50-ml. portions of dioxane. The combined extract was evaporated to 250 ml. with an air stream at room temperature. An aliquot of the extract was diluted 10-fold with dioxane-water (5:1, v/v); duplicate 0.3-ml. aliquots were counted for ^{14}C -radioactivity by mixing with 15 ml. of the toluene-scintillation solution. For chromatography, 10 ml. of the extract was prepared for spotting by the method used previously for urine.

Each blood sample was allowed to thaw in an ice bath and mixed by inversion. A sample of 1.0 ml. was diluted 1:10 with cold distilled water. Duplicate 0.4-ml. aliquots were prepared for radioactivity counting by adding the aliquot to a counting vial containing 1.0 ml. of alcoholic hydrogen peroxide solution (32% aqueous hydrogen peroxide and methanol, 1:3, v/v). The color was allowed to bleach at room temperature for 5 min. before 4 drops were added of a saturated aqueous solution of ascorbic acid. The vials were gently swirled to effect solution, and 15 ml. of the toluene-scintillation solution was added with mixing. The counting samples were virtually colorless and completely dissolved.

The identification and quantitation of the low levels in blood of ^{14}C -PETN and metabolites required pooling of blood samples. For a given time interval after drug administration, 8.0 ml. of blood from each of five subjects was added dropwise to 200 ml. cold dioxane with constant swirling. The homogeneous suspension of blood protein so produced was shaken vigorously for 15 min. and then filtered by vacuum. The residue was washed three times with 50-ml. portions of dioxane. The combined extract and washings were frozen and lyophilized. The dried powder was extracted in the flask four times with 5 ml. of warm methanol. The combined extracts were centrifuged and the supernatant chilled to -20° to induce precipitation of radioactivity-free lipid, which was removed by rapid centrifugation at -5° . The clear supernatant was evaporated to near dryness with an air stream at room temperature. The residue was extracted three times with 1.0 ml. of methanol; the extracts were combined, and 3.0 ml. of distilled water was added to precipitate additional lipid.

After centrifugation, the supernatant was again taken to dryness and the residue extracted with 5.0 ml. of methanol-ethyl acetate

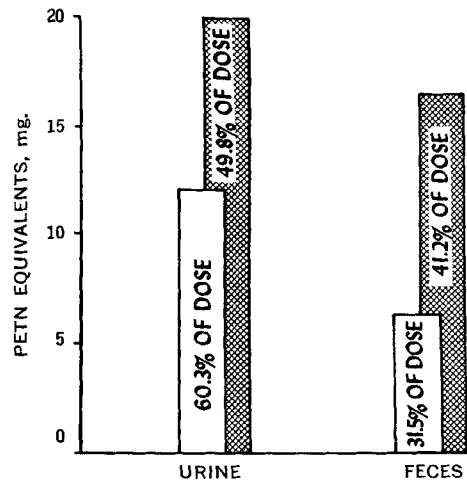


Figure 1—Total urinary (48 hr.) and fecal (72 hr.) excretion of radioactivity administered as ^{14}C -PETN in 20-mg. (□) and 40-mg. (■) doses.

(1:1, v/v). The extract was clarified by centrifugation and the supernatant taken to dryness. This residue was extracted three times with 1.0-ml. portions of methanol, and the combined extracts were reduced to approximately 1.0 ml. with a nitrogen stream. The lipid material was again precipitated by adding 1.0 ml. of distilled water. After centrifugation, the supernatant was evaporated to dryness with a nitrogen stream and the residue taken up in 1.0 ml. of the methanol-ethyl acetate solvent. The solution was clarified by centrifugation and reduced to 200 μ l. for application to a TLC plate. The sample then was applied in an area of 1.5 by 3.0 cm. The plate was developed first in Solvent System K, with an ascending front movement of 13 cm., and then three successive times in Solvent System D with thorough warm air drying between developments. Multiple development with Solvent System D, which consisted of *n*-butanol-ammonium hydroxide-water (4:1:3, upper phase), moved all of the radioactive components into the upper half of the plate ($R_f > 0.5$) and left the bulk of fatty and other interfering material behind. After radioscaning, the silica gel was scraped from the radioactive area of the plate and extracted successively with 2.5, 2.0, 1.5, and 1.0 ml. of warm methanol. The methanol extracts were combined and evaporated to dryness with a nitrogen stream. The radioactive residue was taken up in 3 ml. of methanol, clarified by centrifugation, and reduced to 50–100 μ l. for spotting on a TLC plate. The plate was developed in Solvent System K and scanned to determine the components.

RESULTS

Figure 1 shows the recovery of drug radioactivity following the oral administration of 20- and 40-mg. doses of ^{14}C -PETN. Drug radioactivity was excreted in both urine and feces. The total recovery of administered ^{14}C approximated 92% for both doses. In 48 hr., the equivalent of 20 mg. of PETN or 50% of the 40-mg. dose was excreted, whereas 60% or 12 mg. of the 20-mg. dose was so eliminated. PE and PE mononitrate were the major urinary metabolites, together accounting for 98% of urine radioactivity (Fig. 2). No PETN or PE trinitrate was detected in the urine, and PE dinitrate was present only in very small amounts in the urine for the first few hours after drug administration. For the total 48-hr. excretion, the proportion of urinary PE mononitrate to PE was approximately 1:1 for the low dose and about 3:1 for the high dose.

Fecal elimination of drug radioactivity was extensive and corresponded to 32 and 41% of the 20- and 40-mg. doses, respectively (Fig. 1). The feces contained significant quantities of PETN but no PE trinitrate (Fig. 2). PE was the major drug metabolite in the stool and was accompanied by small amounts of PE dinitrate and PE mononitrate.

Figure 3 illustrates the renal elimination of total drug ^{14}C and of the two major metabolites, PE and PE mononitrate. First-order elimination curves were obtained when the time course of elimination (expressed in molar quantities to account for drug metabolism) was plotted as unexcreted drug radioactivity or individual metabolites, according to the kinetic relationship describing postabsorptive

³ Bio-Solv BBS-3, Beckman Instruments.

⁴ Brinkmann Instruments.

Table I—Renal Elimination of PETN Metabolites

Metabolite	Elimination-Rate Constant, $k \times 10^{-2}$ hr. ⁻¹	Half-Time, hr.	Zero Time Shift, Hr. after Administration
Total ¹⁴ C			
20-mg. dose	9.77 ± 0.50 ^a	7.1	2.0
40-mg. dose	8.40 ± 0.22	8.3	2.1
	$p < 0.02$		
PE			
20-mg. dose	8.55 ± 0.42	8.1	1.1
40-mg. dose	7.82 ± 0.26	8.8	0.8
	$p > 0.10$		
PE mononitrate			
20-mg. dose	11.08 ± 0.55	6.3	2.8
40-mg. dose	8.60 ± 0.38	8.1	2.6
	$p < 0.01$		

^a $k_e \pm$ standard deviation.

and postequilibrative phases of drug excretion and metabolism (3–5):

$$100 \log \left[1 - \frac{A_e}{A_e^\circ} \right] = -k_e t / 2.303 \quad (\text{Eq. 1})$$

where A_e° is the total amount of drug radioactivity (mole equivalents ¹⁴C-PETN) or drug metabolites (moles) excreted after 48 hr., A_e is the cumulative excretion to time t (hr.) after drug ingestion, and k_e is the elimination-rate constant. When absorption, metabolism, and equilibration phases are neglected, the relationship predicts that at $t = 0$ (drug administration) the amount of drug or metabolites distributed in the body equals the drug radioactivity or drug metabolites eventually excreted. Extrapolation of the lines of best fit of the experimental data to $t = 0$ produced values greater than the observed excretion (Fig. 3). Therefore, apparent zero times were calculated from the regression equations for total drug radioactivity and for the PETN metabolites, PE and PE mononitrate. The zero-time shifts were approximately 2.0, 0.9, and 2.7 hr., respectively, after PETN ingestion (Table I). These time periods were not dose dependent.

Table I gives the renal elimination-rate constants and half-times for total PETN radioactivity and also for two constituent major metabolites. Total radioactivity was excreted significantly faster from the 20-mg. than from the 40-mg. dose of ¹⁴C-PETN. This finding is attributed to a difference of PE mononitrate excretion, because there was a significant dose-related difference in the excretion-rate constants of PE mononitrate but not of PE. The faster rate of PE mononitrate excretion (20-mg. dose) was 11.1% of the

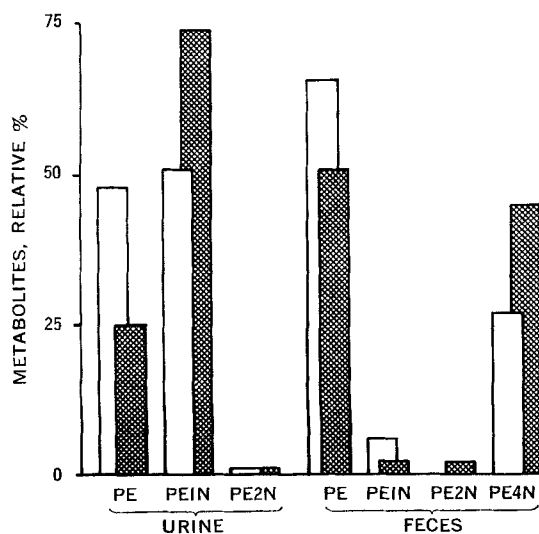


Figure 2—Drug and drug metabolites excreted into the urine (48 hr.) and feces (72 hr.) from 20-mg. (□) and 40-mg. (■) doses of ¹⁴C-PETN. PEIN = PE mononitrate, PE2N = PE dinitrate, and PE4N = PETN.

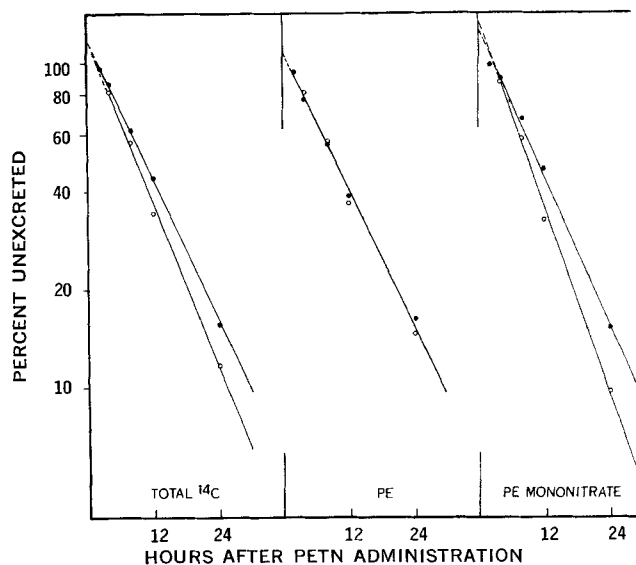


Figure 3—Renal elimination of drug radioactivity and of the two major metabolites after ¹⁴C-PETN administration. The points were calculated from cumulative excretion for 10 subjects (O, 20-mg. dose) and 5 subjects (●, 40-mg. dose). The curves were fitted by the method of least squares.

quantity present in the body per hour, with a half-time of 6.3 hr. The slower rate (40-mg. dose) represented an excretion rate of 8.6% per hour of the body content with a half-time of 8.1 hr. Since the rate constants represent the sum of the rate constants of metabolism and renal excretion (5), the dose-dependent difference in PE mononitrate elimination-rate constant indicated a slower rate of conversion of PE mononitrate to PE for the 40-mg. dose.

Figure 4 shows the blood levels of drug-¹⁴C expressed as μ mole equivalents of PETN per liter of blood. Significant radioactivity was detected within 15 min. of ingestion of both drug doses. Peak blood levels occurred from 4 to 8 hr., with 60% higher levels after the 40-mg. dose. At 2 hr. postadministration, the blood content of administered ¹⁴C accounted for only 4.4 and 3.0% of the 20- and 40-mg. doses, respectively, although approximately 50% of the doses was distributed in the body.

The results obtained by assaying the blood for PETN and its metabolites are also shown in Fig. 4. PETN and PE trinitrate were not detected in the blood. The major metabolite present was PE, with lower levels of PE mononitrate and PE dinitrate. The blood level of these esters was not greatly different for the two doses, and the higher levels of drug-¹⁴C observed with the larger dose was accounted for primarily by PE.

DISCUSSION

The absorption, biotransformation, and excretion of oral doses of 20 and 40 mg. of PETN were qualitatively similar but with notable quantitative differences of clinical interest. Comparative results with the two doses indicated that the absorption of PETN from the intestinal lumen and the subsequent deesterification rate were dose related.

The use of commercial-type tablets of PETN led to more extensive absorption than resulted from the administration of encapsulated ¹⁴C-PETN (6). This finding emphasizes the advisability of performing human metabolic studies with the clinically used dosage form; but even from this dosage form, relatively large quantities of PETN and its metabolite, PE, were found in the feces. This observation inferred either enterohepatic circulation followed by partial excretion of drug and metabolites in the stool or incomplete drug absorption with some intestinal or fecal deesterification. The latter interpretation is favored, because recirculation of PETN would be expected to result in its reduction and hydrolysis, especially by the blood (7) and liver (8); moreover, PE is excreted principally by the renal route (7). Furthermore, PETN is highly insoluble in water or lipid solvents (9), and the appreciable delay of 1–3 hr. for body distribution and equilibration of its metabolites, as determined by their pharmacokinetics (Table I), suggests a slow

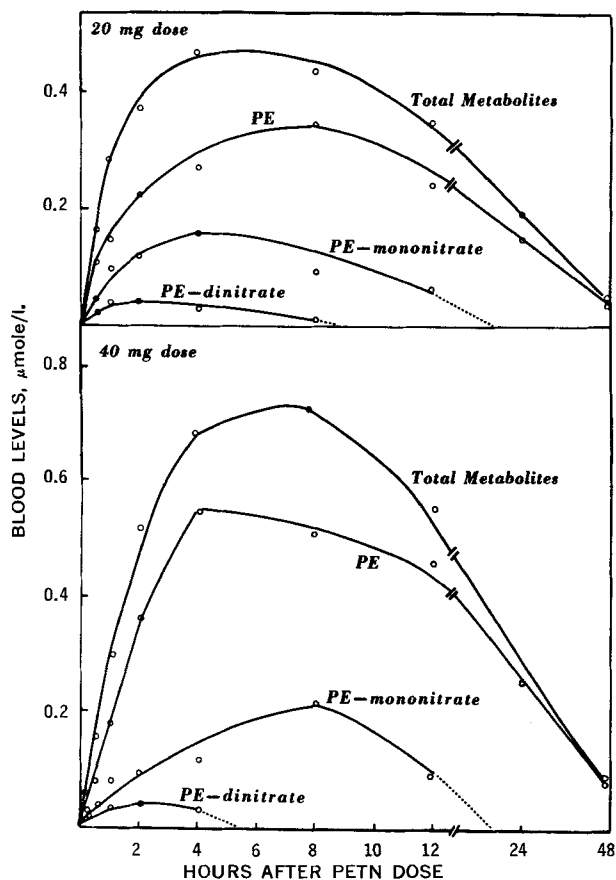


Figure 4—Mean blood levels of total radioactivity and of drug metabolites after 20- and 40-mg. doses of ^{14}C -PETN.

rate of drug absorption. Therefore, it is thought that 60% (12 mg.) of the 20-mg. dose and 50% (20 mg.) of the 40-mg. dose were absorbed, with the remainder excreted in the feces. These values indicate that the absorption process was not saturated and higher doses would result in correspondingly greater absorption.

As in animal species (10–12), PETN is deesterified extensively in man. The absence of PETN and PE trinitrate in the blood and urine suggests that these compounds are degraded more readily than are PE dinitrate and PE mononitrate, a view supported by observations made earlier *in vitro* (7). However, since the blood content of metabolites did not account for more than a few percent of the body content of drug or metabolites, it is also possible that tissue cells contained substantial amounts of the higher esters which were degraded gradually with the release of metabolites into the circulation.

PE mononitrate and PE were virtually the sole metabolites excreted in the urine. The kinetics of their renal excretion indicate that the absorption, body distribution, and equilibration of PETN, and possibly metabolism to PE mononitrate and PE, were complete essentially within 3 hr. after administration (Table I). At this time, which approximated peak blood levels of drug radioactivity, the body pools for these two metabolites closely accounted for the amount of drug absorbed and excreted into the urine as PE mono-

nitrate and PE (Fig. 3). Perhaps the most significant observation, however, was the contrast between the amounts of PE mononitrate and PE excreted into the urine from the 40- and 20-mg. doses (Fig. 2). The amount of PE, the end metabolite, was nearly the same for the two doses, but the amount of PE mononitrate for the high dose was greater than twice that of the lower dose. This finding demonstrates that, in man, there is a limiting rate of deesterification of PE mononitrate. This limiting rate is reflected also by the apparent decrease of the renal elimination rate of PE mononitrate for the 40-mg. dose versus the 20-mg. dose (8.6% hr^{-1} versus 11.1% hr^{-1}), suggesting a decreased rate of metabolism to PE mononitrate. These findings indicate that an accumulation in the body of PE mononitrate, or possibly its precursors, may occur with increasing PETN dosage.

Additionally, if the clinical pharmacology of PETN depends upon the release of nitrite ion, it is unlikely that PE mononitrate contributes significantly because of its slow deesterification. On the other hand, if the PE mononitrate molecule has clinical pharmacological activity, this resistance to degradation may account for the extended duration of action of PETN.

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