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THE ABSORPTION AND BIOTRANSFORMATION OF PENTAERYTHRITOL TETRANITRATE-1,2^{14}C BY RATS

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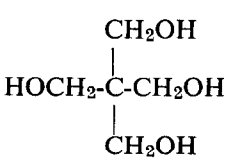
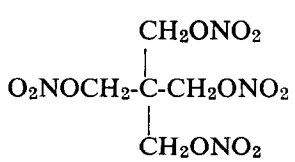
Abstract—Radioactive pentaerythritol tetranitrate (PETN) was administered orally to rats, and its absorption, tissue distribution, and excretion were followed at specified time intervals. By applying thin-layer chromatography and radio-scanning techniques, the four transformation products of PETN were identified and assayed quantitatively in the blood, selected tissues, and excreta. PETN was absorbed at a moderate rate and quickly cleared from the blood by the tissues. The degradation of PETN proceeded in the gastrointestinal tract, in the blood, and in tissues. The extent of PETN degradation was greatest in the liver, spleen, lungs, heart, and blood. Pentaerythritol and its mononitrate were the principal urinary metabolites.

PENTAERYTHRITOL tetranitrate (PETN) is used widely for the relief of angina pectoris and for anginal prophylaxis. In the interest of relating the metabolism of PETN to its pharmacological activity in the same species, the present study represents an effort to account for all of the compound administered, and to do so in terms of metabolites as well as parent drug. The species selected was the rat, and the drug was administered orally since this route is used clinically. At time intervals chosen to cover and to exceed the period of probable pharmacological manifestations, qualitative and quantitative assays were performed on blood, selected tissues, excreta, and the animal carcass. The study was conducted with ^{14}C-labeled PETN, and previously developed methodology[1] was applied for the simultaneous quantitative assay of PETN and its metabolites. In prior investigations,[2, 3] the metabolites of PETN were identified as pentaerythritol (PE) and its mono-, di-, and trinitrates.



Pentaerythritol (PE)



Pentaerythritol tetranitrate (PETN)

MATERIALS AND METHODS

Radioactive PETN. ^{14}C-PE, labeled at C-1 and C-2, was synthesized from acetaldehyde with specific activity of 1·6 mc/m-mole and was employed to prepare ^{14}C-PETN (m.p. 140–141·5°). To minimize the danger of working with explosive material,

the labeled PETN was mixed with seven parts by weight of C.P. lactose. The activity of the lactose–PETN mixture was 0·59 mc/g.

Animals. Female CFN Wistar rats (Carworth Farms) weighing 160–165 g were employed. By gavage, each rat was given a single dose of ^{14}C-PETN (10 mg/kg body weight). Then the animal was placed in an individual glass metabolic unit without food or water.

After withdrawing a blood sample from each animal at a specific time, it was sacrificed immediately. Excised were the heart, lungs, liver, entire gastrointestinal tract (GIT), spleen, kidneys, and adipose tissue from the lumbar and peritoneal regions. Appropriate blood and tissue pools were prepared from six rats. The remaining carcasses were also pooled.

The time periods investigated were 1, 2, 4, and 18 hr post administration. Urine and feces were obtained at all but the first interval, and these collections were pooled by time period as above.

Radioactivity counting. Quantitative assays were conducted with the use of a Packard Tri-Carb model 3003 liquid scintillation spectrometer.

One ml of each urine collection was diluted with 20 ml of scintillation solution and counted directly. The counting efficiency was determined by the external standardization method. The scintillation solution consisted of 7·0 g PPO (2,5-diphenyloxazole), 0·3 g dimethyl POPOP [1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene], and 100·0 g naphthalene in 1·0 l. redistilled dioxane.

One ml of each blood pool was diluted to 50 ml with distilled water, and 1·0 ml of this dilution was mixed with 20 ml scintillation solution for counting.

The carcass and tissue pools were homogenized in a Waring Blendor with aqueous dioxane (75 per cent). The homogenates were filtered, and the residues were re-extracted with 75 per cent dioxane. The volumes of solvent employed were 1500 ml for carcasses and 200 ml for tissues. One milliliter of each extract was mixed with 20 ml scintillation fluid for counting.

Thin-layer chromatography. Glass plates were employed, measuring 2 × 8in. and coated with 250 μ of silica gel G bound with calcium sulfate. The unidimensional ascending technique was used to develop the chromatograms in the following solvent systems: D, *n*-butanol:ammonium hydroxide:water (4:1:3, upper phase); J, toluene:ethyl acetate:*n*-butanol:water (10:5:2:2, upper phase). The developed chromatograms were scanned for radioactivity with a Packard model 7200 radiochromatogram scanner. The area under each peak was determined with a Keuffel & Esser compensating polar planimeter. In this manner, the R_f value and the relative quantity of each component were obtained.

The R_f values obtained in solvent D are PE, 0·26; and nitrate esters, 0·6–0·8. In solvent J, the R_f values are PE, 0·00; PE-mononitrate, 0·16–0·23; PE-dinitrate, 0·45–0·55; PE-trinitrate, 0·60–0·69; and PETN, 0·77–0·84. After scanning each plate developed in solvent J, the plate was redeveloped in solvent D to confirm the identity of PE. The identification of these five compounds were based upon comparative chromatography in several solvent systems, and fundamentally upon the carbon and nitrate contents of eluates as described earlier.[1]

Preparation of samples for chromatographic assay. Aliquots of the urines containing up to 0·2 μc ^{14}C were evaporated to dryness in a stream of air at room temperature. The residues from the evaporation were taken up in 1·0 ml of methanol:ethyl acetate

(1:1, v/v), then concentrated to a minimum volume for thin-layer chromatography in solvent J.

The blood and other tissue samples contained significant amounts of lipids which interfered with the concentration of the metabolites to a suitable level and with the resolution of the metabolites on the chromatogram. The concentration of these samples required considerably more manipulation to remove the interfering materials with a minimum loss of activity. The blood samples were shaken with 50 ml of 75 per cent dioxane and filtered; the residues were re-extracted with dioxane and the entire extract used for concentration. In the case of the other tissues, either the entire dioxane extract was used or aliquots containing not more than 0·2 μc were taken.

The dioxane was removed by evaporation in a stream of air at room temperature. The residue from the evaporation was taken up in several (3–8) 5-ml portions of methanol. The insoluble material was discarded and the methanol evaporated. If the residue from the evaporation of the methanol was excessive ($>$ 0·5 g), the methanol extraction was repeated with not more than three 5-ml portions of methanol. The residue was extracted with 5 ml methanol, centrifuged to remove insolubles, diluted with 0·5 ml water, centrifuged to remove precipitated lipids, diluted to 10 ml with water, and centrifuged once more. The supernatant solution was evaporated again, taken up in 1 ml of methanol:ethyl acetate (1:1, v/v) and, after clarifying by centrifugation, an aliquot was counted to check losses to this stage. If the sample contained less than 0·04 μc at this stage, as was the case with blood samples, further removal of lipids was accomplished by dissolving the material in 1 ml methanol and precipitating with 1 ml water, followed by resolution in methanol–ethyl acetate.

The methanol–ethyl acetate solution was concentrated to less than 0·5 ml and spotted on a chromatographic plate as a thin streak and developed in solvent J for assay of the metabolites. If, as was usually the case, the sample could not be applied in a narrow band, or if on development the solvent would not percolate through the spot, the sample was applied to the plate over an area of up to 1·5 $\times$ 3 cm, then developed first in solvent J followed by three successive developments in solvent D. This operation, which moves all the activity near the solvent front, leaving the bulk of the interfering materials in the lower portion of the plate, was generally found to be necessary if the total amount of material exceeded about 0·03 ml. The inactive silica gel was removed from the plate and the activity eluted from the remainder with methanol; the effectiveness of the elution was determined by scanning. The eluted sample was concentrated and reapplied to a fresh plate. The bloods and some of the tissues required a repetition of the multiple-development procedure in solvent D before a satisfactory chromatogram could be obtained on a third plate. Tracer studies with PETN added to urine, blood, and various tissues showed that no significant degradation resulted from the manipulations just described.

RESULTS

Gastrointestinal tract

From the data presented in Table 1 it is evident that 7 to 8 per cent of the ^{14}C-PETN was absorbed during the first hour. Since ^{14}C was not recovered quantitatively at the subsequent time intervals, the minimal amounts of ^{14}C absorbed were estimated (from the difference between total recovery and GIT content) as 14 per cent after 2 hr, 24 per cent after 4 hr, and 60 per cent after 18 hr.

Feces

^{14}C was first found in the feces 2 hr after administration; the amount was 0·16 per cent of the dose. After 4 hr, the quantity of defecated ^{14}C had doubled. Subsequently, the rate increased, and 10 per cent of the dose was eliminated in the feces by the end of 18 hr.

TABLE 1. DISTRIBUTION OF ^{14}C AFTER ORAL ADMINISTRATION OF ^{14}C-PETN TO RATS

	Radioactivity found after							
	1 hr		2 hr		4 hr		18 hr	
Specimen	(μg/g)*	(% of dose)	(μg/g)*	(% of dose)	(μg/g)*	(% of dose)	(μg/g)*	(% of dose)
Blood†	0·58	0·58	0·67	0·67	0·69	0·69	0·31	0·31
GIT	83·57	92·86	73·98	79·60	84·04	70·86	51·49	33·84
Heart	0·66	0·04	0·56	0·03	0·59	0·04	0·31	0·02
Kidney	1·39	0·14	2·06	0·20	2·22	0·22	6·99	0·73
Liver	1·25	0·56	2·79	1·32	1·96	0·88	2·72	0·99
Lung	0·61	0·06	0·70	0·08	0·89	0·10	0·41	0·04
Spleen	0·92	0·02	4·92	0·11	6·06	0·15	5·74	0·13
Adipose	1·30	1·29	3·41	0·84	4·38	0·99	9·94	3·08
Carcass	0·68	5·63	1·28	10·71	1·54	12·56	1·96	16·76
Urine				0·35		8·33		28·23
Feces			4·93	0·16	14·66	0·29	217·04	10·13
Total recovery		101·18		94·07		95·11		94·26

* Expressed as μg equivalents of PETN/g tissue or body fluid assayed.
† Blood was assumed to constitute 10 per cent of body weight.

Blood

From Table 1 it is evident that the ^{14}C blood level remained in the same range over the period from 1 to 4 hr post administration. Qualitatively, however, the picture was quite different (Table 2). At 1 hr, there was little PE-dinitrate in the blood, but this level increased to 28 per cent of the radioactivity at 4 hr. PE-Mononitrate was at about the same level from 1 through 4 hr, and was the only nitrate present at 18 hr. PETN and PE-trinitrate were not detected in blood at any time interval studied.

Kidneys, urine

At 1 hr, the ^{14}C content of the kidneys was more than twice as high as the blood level (Table 1). From 2 to 4 hr, the ratio increased to 3 to 1, and at 18 hr it was more than 20 to 1. The rate of urinary excretion, 28 per cent of the ^{14}C in 18 hr, is comparable to data obtained with mice[4, 5] and humans.[6]

Heart, lungs

The ^{14}C concentrations in the heart and lungs were similar over the entire period of study (Table 1). Furthermore, the composition of the radioactive mixtures was quite similar (Table 3). Comparison of the metabolites present in blood and heart shows consistently higher blood levels of PE-mononitrate and consistently higher heart levels of PE.

Liver, spleen

Throughout the experiment, the ^{14}C concentrations in the liver and spleen exceeded the blood levels. At 1, 2, and 4 hr, the liver level was about three times greater than

TABLE 2. PETN BIOTRANSFORMATION AS REFLECTED BY METABOLITES IN BLOOD, GIT AND EXCRETA

Specimen	Time	PETN		PE-Trinitrate		PE-Dinitrate		PE-Mononitrate		PE	
		(μg/rat)*	(%)†	(μg/rat)*	(%)†	(μg/rat)*	(%)†	(μg/rat)*	(%)†	(μg/rat)*	(%)†
Blood	1					0·18	2·0	5·64	60·8	3·48	37·3
	2					1·33	12·4	6·50	60·6	2·89	27·0
	4					3·05	27·6	5·99	54·3	2·00	18·1
	18							1·08	23·5	3·50	76·5
GIT	1	1242	83·3	84	5·6	51	3·4	57	3·8	57	3·8
	2	692	53·8	170	13·2	113	8·8	127	9·9	184	14·3
	4	670	59·1	122	10·8	60	5·3	74	6·5	208	18·3
	18	22	4·3					11	2·2	475	93·5
Urine	2					4·3	32·5	6·85	51·8	2·08	15·7
	4					10·4	7·8	35·5	26·6	87·4	65·6
	18							119	26·9	324	73·1
Feces	2							0·10	4·0	2·46	96·0
	4							0·13	2·8	4·56	97·2
	18	8·0	5·3							144	94·7

* Expressed as μg equivalents of PETN/rat.
† Per cent of total radioactivity in the specimen.

TABLE 3. PETN AND METABOLITES FOUND IN RAT TISSUES

Tissue	Time	PETN		PE-Trinitrate		PE-Dinitrate		PE-Mononitrate		PE	
		(μg/rat)*	(%)†	(μg/rat)*	(%)†	(μg/rat)*	(%)†	(μg/rat)*	(%)†	(μg/rat)*	(%)†
Heart	1					0·06	10·0	0·29	50·0	0·23	40·0
	2							0·13	25·9	0·38	74·1
	4	0·09	16·3	0·04	7·3	0·02	4·1	0·09	16·3	0·31	56·1
	18									0·23	100·0
Kidney	1							0·72	31·5	1·57	68·5
	2					0·15	4·8	1·43	45·2	1·58	50·0
	4							0·31	8·8	3·18	91·2
	18							0·30	2·7	10·67	97·3
Liver	1							2·20	24·4	6·81	75·6
	2					0·88	4·2	10·59	50·6	9·46	45·2
	4									14·03	100·0
	18									14·88	100·0
Lung	1					0·02	2·5	0·42	43·0	0·54	54·5
	2					0·08	6·7	0·34	28·6	0·77	64·7
	4	0·18	11·4	0·09	5·7	0·08	5·0	0·30	18·6	0·96	59·3
	18									0·69	100·0
Spleen	1					0·03	8·9	0·10	268	0·24	64·3
	2			0·07	3·7	0·45	24·8	0·72	39·8	0·58	31·8
	4							0·47	19·7	1·91	80·3
	18	0·04	2·0			0·02	1·0	0·18	8·9	1·77	88·1

* Expressed at μg equivalents of PETN/rat.

† Per cent of total radioactivity in the specimen.

the blood level, and at 18 hr it was nine times greater. The concentration of ^{14}C by the spleen was even more pronounced; the ratios were roughly 2, 7, 9, and 19 at four time intervals. The proportions of PE in liver and spleen exceeded the blood values. At 4 and 18 hr, 100 per cent of the ^{14}C was present as PE in the liver, and the corresponding values for spleen were 80 and 88 per cent.

Fat

The concentration of ^{14}C in the adipose tissue always exceeded the blood level, and climbed throughout the experiment. The fat/blood proportions were approximately 2 at 1 hr, 5 at 2 hr, 6 at 4 hr, and 33 at 18 hr. Some PE-trinitrate was found in fat, and the concentration was about the same at 18 hr as it was after 1 and 2 hr.

Carcass

Radioactivity from ^{14}C-PETN increased in the carcass and amounted to 17 per cent of the dose at 18 hr after administration (Table 1). From Table 3 it is evident that the carcass contained the largest quantities of PE-mono-, di- and trinitrates.

DISCUSSION

The rate of PETN absorption was maximal during the second hour after oral administration. This observation may be attributed to extensive passage into the small intestine,[7] a consideration consistent with the elimination of a small quantity of radioactive material into the feces between the first and second hours. More outstanding than the moderate absorption rate was the rapid clearance of radioactive compounds from the blood; more than 90 per cent of the radioactivity absorbed during the first hour was already cleared from the blood and located in the tissues. Of the radioactivity in the tissues at that time, 90 per cent was found in the fat and carcass, and PE nitrates accounted for about 75 per cent of this radioactivity. By contrast, the kidney contained only 2 per cent of the tissue radioactivity, and almost 70 per cent of it was present as PE.

In connection with the recovery of 94–95 per cent of the administered radioactivity at periods of 2 hr and longer (Table 1), it should be pointed out that PE is the major final metabolite of PETN in the rat. A separate study[8] showed that less than 0·3 per cent of the ^{14}C-PETN administered to rats was eliminated as $^{14}CO_2$ in 24 hr. This small quantity may reflect bacterial degradation in the intestine and/or the presence of a radioactive impurity in the PETN employed.

The changing composition of the urine reflected the increasingly extensive metabolism of PETN. The first urine collection (at 2 hr) contained PE-mononitrate as the main metabolite and had twice as much PE-dinitrate as PE. At 4 hr, PE was the major urinary metabolite, and at 18 hr no PE-dinitrate was present.

Previous studies[2, 3] showed that PETN and PE-trinitrate were degraded readily by blood *in vitro*, but that the dinitrate and mononitrate were attacked more slowly. From the present investigation, it appears that this situation may also obtain *in vivo*, and be complicated by the rapid clearance of organic nitrates from the blood. It seems also that certain organs may be better equipped than blood to convert PE-mononitrate into PE. These organs are the heart, lung, liver, and spleen. Although the experimental data may be interpreted to make the same case for the kidney, it seems probable that the kidney may preferentially collect PE from blood.

The study of PETN metabolism in the rat has yielded satisfactory quantitation between the administered and recovered radioactivity. The data are expected to be of value in interpreting pharmacological findings in the same species in terms of (1) the onset and duration of effects and (2) the specific metabolites present.

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