Biochemical Pharmacology, Vol. 18, pp 1985–1990. Pergamon Press. 1969. Printed in Great Britain

PENTAERYTHRITOL TRINITRATE METABOLISM BY THE RAT

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(*Received* 15 *January* 1969; *accepted* 28 *February* 1969)

Abstract—Rats were studied after being given a single dose of ^{14}C-pentaerythritol trinitrate by gavage. The labeled components of the blood, urine and gastrointestinal tract were identified by thin-layer chromatography and assayed quantitatively by radioscanning. Pentaerythritol trinitrate was found to be absorbed and excreted rapidly by the rat. The drug metabolites were pentaerythritol, pentaerythritol mononitrate and pentaerythritol dinitrate. Comparisons of the data with the results of similar studies with pentaerythritol tetranitrate (PETN) and nitroglycerin showed that pentaerythritol trinitrate was absorbed much faster than PETN and almost as quickly as nitroglycerin, and that the urinary excretion of pentaerythritol trinitrate proceeded at the highest rate. Additionally, pentaerythritol trinitrate was the only one of the three drugs which passed intact into the urine.

PENTAERYTHRITOL trinitrate was first identified as a metabolite of pentaerythritol tetranitrate (PETN) during the course of studies with human blood.[1] Subsequently this metabolite was found to be formed from PETN administered to rats[2–5] and humans,[6] and also to be produced from PETN by hog liver enzyme,[3] rat blood erythrocytes,[7] mouse liver parenchymal and Kupffer cells[8] and rat heart mitochondria and microsomes.[9] Since pentaerythritol trinitrate is far more soluble than PETN in water and organic solvents,[10] we were interested in learning whether its metabolism might resemble that of nitroglycerin.[11]

MATERIALS AND METHODS

^{14}C-pentaerythritol trinitrate. This compound was synthesized from pentaerythritol-1,2-^{14}C at the Hercules Research Center. Assay by TLC[12] indicated the product to contain 97·3% pentaerythritol trinitrate, 2·1% PETN and 0·6% pentaerythritol. The synthetic preparation was dispersed on 9 parts by weight of C.P. lactose. The specific activity of the pentaerythritol trinitrate–lactose mixture was 0·302 mc/g.

Animals. Groups of three female CFN Wistar rats (Carworth Farms) weighing 200–210 g were used. Each rat was given a single dose of ^{14}C-pentaerythritol trinitrate (10 mg/kgbody wt.) by gavage and placed into an individual glass metabolic unit without food or water.

At a specific interval after drug administration, a blood sample was withdrawn and the animal was sacrificed immediately. Then the heart, lungs, liver, kidneys, spleen, entire gastrointestinal tract (GI tract) and a 1-g sample of adipose tissue from

the lumbar region were excised. The blood and tissue samples were pooled appropriately. Also pooled were the remaining carcasses.

The time periods studied were 1-, 2-, 4- and 18-hr post-administration. The urine collections were kept separate, but the fecal collections were pooled by time period.

Radioactivity counting. A Packard Tri-Carb model 3324 liquid scintillation spectrometer was used for quantitative assays.

One ml of each urine collection was diluted with 18 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-phenyl-oxazolyl)-benzene], and 100·0 g naphthalene in 1·0 l. redistilled dioxane.

One ml of each blood was diluted to 50 ml with distilled water, and 1·0 ml of this dilution was mixed with 18 ml scintillation solution for counting.

The carcass, feces and tissue pools were homogenized in a Waring blendor with aqueous dioxane (75%). The homogenates were filtered, and the residues were re-extracted thoroughly with 75% dioxane. The volumes of solvent employed were approximately 4 l. for carcasses, 1 l. for the GI tracts and livers, 200 ml for the other tissues and 200 ml to 1 l. for the feces. One ml of each extract was mixed with 18 ml scintillation fluid for counting.

In an effort to account for all of the administered radioactivity, portions of the extracted livers, GI tracts and carcasses were digested with Hyamine. Tissue samples (10–50 mg) were placed into scintillation vials containing 0·5 ml of water and 1·5 ml of hydroxide of Hyamine 10X and the vials were shaken in a water bath at 37° for 18 hr. Then the scintillation solution was added to count the digestion mixtures.

Thin-layer chromatography. Glass plates were employed, measuring 2 × 8 in. and coated with 250μ of silica gel G bound with calcium sulfate. The unidimensional ascending technique was used to develop the chromatograms in 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 and 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 were pentaerythritol, 0·00; pentaerythritol mononitrate, 0·16–0·23; pentaerythritol dinitrate, 0·45–0·55; and pentaerythritol trinitrate, 0·60–0·69.[6] The identification of these four compounds was based upon comparative chromatography in several solvent systems, and fundamentally upon the carbon and nitrate contents of eluates as described earlier.[12]

RESULTS

The oral administration of radioactive pentaerythritol trinitrate to rats resulted in high levels of blood radioactivity for 4 hr. The data are shown in Table 1 and are compared with nitroglycerin and PETN blood levels in Fig. 1. The relative rates of absorption of these organic nitrates are also reflected by their removal from the GI tract (Fig. 2). Both views indicate pentaerythritol trinitrate to bear more resemblance to nitroglycerin than to PETN. The same statement applies to urinary excretion (Fig. 3). Radioactivity from ^{14}C-pentaerythritol trinitrate quickly appeared in the

TABLE 1. DISTRIBUTION OF ^{14}C AFTER ORAL ADMINISTRATION OF ^{14}C-PENTAERYTHRITOL TRINITRATE 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†	2·95	2·95	1·78	1·78	1·42	1·42	0·13	0·13
GI tract	59·8	46·30	59·8	48·57	51·7	34·67	25·8	15·57
Heart	13·8	0·53	2·22	0·07	1·20	0·04	0·14	0·003
Kidneys	14·5	0·98	6·61	0·43	4·02	0·27	0·65	0·04
Liver	11·9	3·24	5·29	1·82	3·48	1·14	0·50	0·13
Lungs	4·39	0·44	2·63	0·16	1·76	0·10	0·28	0·02
Spleen	7·81	0·11	3·94	0·07	1·80	0·03	0·60	0·01
Adipose	6·19	0·38	4·03	0·26	4·66	0·27	2·28	0·11
Carcass	4·67	40·08	3·16	25·26	2·11	16·93	1·05	8·14
Urine		6·49		19·37		35·73		47·53
Feces		0·01		0·61		7·29		17·86
Total recovery		101·51		98·40		97·89		89·54

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

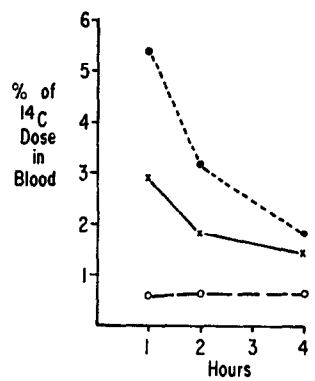

FIG. 1. Comparison of the radioactivity in the blood of rats given the same dose of nitroglycerin, ●; pentaerythritol trinitrate, ×; and PETN, ○.

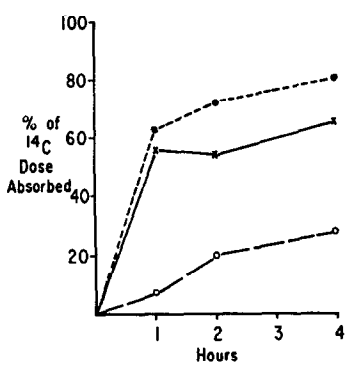

FIG 2. Comparison of the rates of absorption of nitroglycerin, ●; pentaerythritol trinitrate, ×; and PETN, ○, from the gastrointestinal tract of rats.

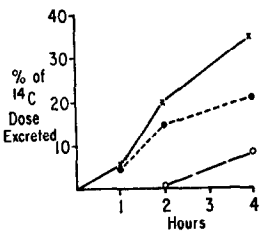

FIG. 3. Comparison of the urinary excretion of radioactivity by rats administered ^{14}C-pentaerythritol trinitrate, ×; ^{14}C-nitroglycerin, ●; and ^{14}C-PETN, ○.

urine and 6·4 per cent of the dose was so eliminated within an hour after administration; by 18 hr almost half of the dose was passed into the urine (Table 1). As observed with PETN, a significant quantity of the ^{14}C from pentaerythritol trinitrate was excreted with the feces.

The data presented in Table 1 also show that radioactivity was cleared from the blood and reached higher concentrations in the tissues. These ^{14}C-tissue levels decreased with time. This release is notably different from the PETN results, particularly with respect to the fat and carcass. After ^{14}C-PETN administration, the ^{14}C concentration in the carcass and adipose tissue increased steadily. From 1 hr to 18 hr,

TABLE 2. PENTAERYTHRITOL TRINITRATE METABOLISM AS INDICATED BY DEGRADATION PRODUCTS IN THE GI TRACT, BLOOD AND URINE

Specimen	Time (hr)	PE-trinitrate		PE-dinitrate		PE-mononitrate		PE	
		(μg/rat)*	(%)†	(μg/rat)*	(%)†	(μg/rat)*	(%)†	(μg/rat)*	(%)†
GI tract	1	312	29·5	180	17·0	121	11·4	444	42·0
	2	276	27·3	112	11·1	82	8·1	541	53·5
	4	81	10·9	47	6·3	70	9·4	544	73·4
	18	0	0·0	0	0·0	0	0·0	339	100·0
Blood	1	0·42	0·7	2·4	4·0	17·2	28·1	41·0	67·2
	2	0·28	0·7	0·6	1·7	6·2	15·8	31·8	81·8
	4	0·08	0·3	1·4	5·2	7·6	30·0	16·6	64·5
	18	0·0	0·0	0·0	0·0	0·18	8·0	2·16	92·0
Urine	1	3·8	2·1	45·8	25·6	19·2	10·7	110	61·6
	2	0·4	0·1	25·8	6·4	38·7	9·6	339	83·9
	4	0·0	0·0	49·8	7·7	95·8	14·8	501	77·5
	18	0·0	0·0	154	15·5	227	22·8	614	61·7

* Expressed as μg equivalents of pentaerythritol trinitrate per rat.
† Per cent of total administered radioactivity in the specimen.

the concentration of ^{14}C in fat tissue and in the carcass increased about 8-fold and 3-fold respectively. With pentaerythritol trinitrate over the same time interval, the ^{14}C concentrations decreased by about 63 per cent in the fat and 78 per cent in the carcass. The situation with nitroglycerin is not comparable because this drug was

converted into glycerol which subsequently entered into normal metabolic pools.[11, 13]

The data obtained by quantitatively assaying the GI tract, blood and urine for pentaerythritol trinitrate and its metabolites are presented in Table 2. Like PETN, pentaerythritol trinitrate was degraded during its residence in the GI tract. By 18-hr postadministration, there was no pentaerythritol trinitrate in the tract; pentaerythritol was the only tagged compound present. Significant quantities of unaltered pentaerythritol trinitrate were found in blood for 4 hr after administration. This constitutes another major difference from PETN metabolism; neither PETN nor pentaerythritol trinitrate was detected in the blood at any time from 1 hr to 18 hr after PETN administration. Still another notable difference was the urinary excretion of unaltered pentaerythritol trinitrate after its administration in contrast to the absence of both PETN and pentaerythritol trinitrate in the urine after dosing with PETN. In this respect, pentaerythritol trinitrate also differed from nitroglycerin which was not detected as such in urine collected as early as 30 min after drug administration.

DISCUSSION

This first metabolic study of pentaerythritol trinitrate shows distinct differences from PETN. The faster oral absorption and slower de-esterification of the trinitrate are metabolic characteristics which may presage clinical advantages over PETN and other organic nitrates. Indeed, the rats handled pentaerythritol trinitrate more like nitroglycerin than PETN. As expected, the degradation of pentaerythritol trinitrate proceeded through the intermediate nitrates to pentaerythritol. Some of this degradation is considered to occur in the GI tract as was observed earlier with PETN.[14] At this time, however, it is not known whether the pentaerythritol nitrates enter the enterohepatic circulation or whether the drug metabolites found in the tract result exclusively from the action of the bacterial flora. With respect to their ultimate fate, the pentaerythritol nitrates yield the refractory end product, pentaerythritol, whereas nitroglycerin is converted into carbon dioxide, organic acids,[11] lipids, proteins, glycogen, RNA and DNA.[13]

Acknowledgements—The authors are grateful to Drs. H. L. Young and L. R. Kangas of Hercules Inc. for their synthesis of ^{14}C-pentaerythritol trinitrate. We also appreciate the assistance of Dr. R. I. Meltzer in obtaining this preparation and the technical assistance of Mr. E. J. Merrill.

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