

## Quantitative Recovery of Radioactivity from $^{14}\text{C}$ -Pentaerythritol Tetranitrate Administered to Rats

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A single dose of  $^{14}\text{C}$ -labeled pentaerythritol tetranitrate (PETN) was administered orally to fasted rats, and the level of radioactivity was determined 24 hr. later in the blood, liver, gastrointestinal tract (GIT), urine, and carcass. One group of treated rats was housed to permit collection of expired  $\text{CO}_2$  which was also assayed for  $^{14}\text{C}$ . It was possible to account for all of radioactivity administered. More than 40 per cent of the drug was absorbed, and about 25 per cent of the  $^{14}\text{C}$  was excreted into the urine. The blood, GIT, and urine, were also assayed quantitatively for PETN and its metabolites. The blood contained pentaerythritol and its mono- and dinitrates. Urine contained these metabolites and also a trace of pentaerythritol trinitrate. The GIT contained PETN and pentaerythritol.

A PREVIOUS STUDY (1) examined the absorption and subsequent metabolism of  $^{14}\text{C}$ -pentaerythritol tetranitrate (PETN) administered in ligated sections of the rat gastrointestinal tract. The present experiment was undertaken to evaluate the necessity of monitoring the respiratory carbon dioxide in a more general metabolic study of normal (*i.e.*, nonligated) rats. This experiment provided, furthermore, the opportunity to evaluate the efficacy of the extraction methods used in the estimation of PETN and its metabolites.

In the metabolism of PETN the nitrate groups are removed to form PE-trinitrate, PE-dinitrate, PE-mononitrate, and finally pentaerythritol (PE); no breakdown products from the PE have been found (2-5). Although no evidence of expired  $^{14}\text{CO}_2$  was found in a comparable study using mice (3), this determination was necessary in rats to plan the procedures for animal management in a more complete metabolic study.

For the present experiment,  $^{14}\text{C}$ -PETN was administered to six rats housed in glass metabolic cages in such a manner as to provide for the collection of the expired carbon dioxide. After 24 hr. the rats were sacrificed and the  $^{14}\text{C}$  content of the carbon dioxide assayed. The blood and some of the tissues were extracted, and the radioactivity of both the extract and the residue was measured to evaluate the efficacy of the extraction. The distribution of the metabolites of PETN in the extracts and the urine was also determined.

### METHODS

**Radioactive PETN.**— $^{14}\text{C}$ -PE labeled at C-1 and C-2 was synthesized from acetaldehyde with specific activity of 1.6 mc./mmole and was employed to prepare  $^{14}\text{C}$ -PETN, m.p. 140-141.5°. To minimize the danger of working with explosive material, the labeled PETN was mixed with 7 parts by weight

of chemically pure lactose. The activity of the lactose-PETN mixture was 0.59 mc./Gm.

**$\text{CO}_2$  Collection.**—Six white female Wistar rats weighing approximately 180 Gm. each were fasted for a period of 24 hr. prior to the experiment. The rats were dosed orally with 1.8 ml. of a 8.0 mg./ml. suspension of PETN-lactose in propylene glycol (10 mg.  $^{14}\text{C}$ -PETN/Kg. body weight) and housed in glass metabolic cages connected to a gas absorption train.

The gas absorption train consisted of a drying tower containing Drierite and Ascarite connected to a source of low-pressure air, followed by a gas-scrubber containing water for rehumidifying the air. The air from the scrubber was passed to a manifold to distribute the air to the six metabolic cages. The effluent air from the cages was recombined in a second manifold and bubbled successively through three containers of 10% sodium hydroxide to trap the expired  $\text{CO}_2$  before exhaustion to the atmosphere. A total of 4 L. of 10% NaOH was used in the three collection flasks.

After 24 hr. of  $\text{CO}_2$  collection, the combined NaOH solutions were treated with a saturated solution of barium chloride until the precipitation of barium carbonate was complete and crystallization of barium hydroxide was evident. The precipitate was filtered, washed with 4 L. of water, and dried.

The animals were removed from the cages and sacrificed immediately. The blood, liver, gastrointestinal tract, and carcass were extracted with dioxane, and the residues from the extractions assayed for  $^{14}\text{C}$  by combustion analysis. The barium carbonate was assayed by gas evolution analysis.

**Radioactivity Counting.**—Quantitative assays of the urine and the dioxane extracts for  $^{14}\text{C}$  were conducted by scintillation spectrometry in a Packard Tricarb using a dioxane solvent for the scintillation solution. The combustion analyses of the residues and the gas evolution analysis of the barium carbonate were conducted by the New England Nuclear Corp.

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**Thin-Layer Chromatography.**—The assay of the urine and the dioxane extracts for metabolites of PETN was carried out by means of thin-layer chromatography and radioscanning as previously described (1).

### RESULTS AND DISCUSSION

Several hours prior to the expiration of the experiment one of the six rats died. The urine and tissue used for this study were, therefore, taken only from the five survivors. The results of the assay for total  $^{14}\text{C}$  are given in Table I. The distribution of PETN metabolites in the blood, urine, and gastrointestinal tract is given in Table II. The carcass extract was not assayed qualitatively. The liver extract proved too intractable to produce a satisfactory chromatogram.

TABLE I.—RECOVERY OF  $^{14}\text{C}$  24 hr. AFTER ORAL ADMINISTRATION OF  $^{14}\text{C}$ -PETN

Sample	Dose, %		Total
	Extract	Residue	
Blood	0.61	0.02	0.63
Liver	0.24	0.08	0.32
GIT	54.73	3.92	58.65
Urine	25.21	—	25.21
Carcass	15.73	1.20	16.93
$\text{CO}_2^a$	0.28	—	0.28
	96.8	5.2	102.0

<sup>a</sup> Value from "5.5" rats.

TABLE II.—DISTRIBUTION OF PETN METABOLITES AT 24 hr. POSTADMINISTRATION

Sample	Compn., % <sup>a</sup>				PETN
	PE	PE-Mono-nitrate	PE-Di-nitrate	PE-Tri-nitrate	
Blood	45	27	28	—	—
GIT	58	—	—	—	42
Urine	51	30	17	2	—

<sup>a</sup> Calculated on a molar basis.

The small amounts of radioactivity found in the residues from the tissue extraction confirm the efficacy of the extraction procedure. The one notable exception is the liver extraction which removed only 75% of the radioactivity from the tissue.

The barium carbonate collected weighed 108.6 Gm. A blank with the apparatus using no rats produced 2.9 Gm. of barium carbonate. The difference of 105.7 Gm. of barium carbonate, corresponding to 23.6 Gm. of carbon dioxide, represents the air expired by "5.5" rats in the 24 hr. On this basis it is estimated that each young rat (fasted for 48 hr. as described) exhaled about 24 Gm. of carbon dioxide per kilogram body weight. This quantity is

in agreement with data reported by Benedict and MacLeod (6).

From the finding (Table I) that the rats exhaled  $^{14}\text{CO}_2$  to the extent of only 0.28% of the  $^{14}\text{C}$ -PETN administered, it is evident that there was no significant conversion of PETN or its metabolites to carbon dioxide. This observation confirms the finding in mice (3). One possible source of the  $^{14}\text{CO}_2$  might have been some trace quantity of radioactive impurity in the PETN. Another possibility is that the bacterial flora of the intestine degraded a small quantity of pentaerythritol. There seems to be no information on this point. In their review on the metabolism of tetritols, Carr and Krantz (7) indicated that some microorganisms degrade erythritol. Erythritol, of course, contains two secondary alcohol groups which may render it more vulnerable to enzymatic attack than is the completely symmetrical, primary alcohol-containing pentaerythritol.

Kutscher (8) studied the chronic feeding of pentaerythritol to rats and reported almost 90% of the compound to have been excreted without structural alteration. Considering that Kutscher did not employ radioactive material, his recovery was very high, and inclines one to accept the generalization (9) that compounds containing four alcohol groups are not metabolized by mammals.

More than one-half of the radioactivity of the initial dose was found in the gastrointestinal tract. Of this activity one-third was PETN and two-thirds pentaerythritol; none of the intermediate organic nitrates was detected. One might speculate that the PETN was located in the upper intestinal tract since it was shown previously (1) that the large intestine or its flora is capable of a measurable degradation of the PETN which would result in the detection of the intermediates. The pentaerythritol in the gastrointestinal tract may be the result of this action in the large intestine, although it could be found in the small intestine as a result of bile recirculation or in the stomach as a result of coprophagy. Since the design of the metabolic cages permitted coprophagy, it is not surprising that after 48 hr. of starvation, there were no feces available for collection.

### REFERENCES

- (1) DiCarlo, F. J., Coutinho, C. B., Crew, M. C., Sklow, N. J., and Haynes, L. J., *Angiology*, to be published.
- (2) DiCarlo, F. J., Hartigan, J. M., Jr., and Phillips, G. E., *Life Sciences*, **3**, 563(1964).
- (3) DiCarlo, F. J., Hartigan, J. M., Jr., Coutinho, C. B., and Phillips, G. E., *Proc. Soc. Exptl. Biol. Med.*, **118**, 311 (1965).
- (4) DiCarlo, F. J., Hartigan, J. M., Jr., and Phillips, G. E., *ibid.*, **118**, 514(1965).
- (5) DiCarlo, F. J., Coutinho, C. B., Sklow, N. J., Haynes, L. J., and Crew, M. C., *ibid.*, **120**, 705(1965).
- (6) Benedict, F. G., and MacLeod, G., *J. Nutr.*, **1**, 343 (1929).
- (7) Carr, C. J., and Krantz, J. C., Jr., in "Advances in Carbohydrate Chemistry," vol. 1, Academic Press Inc., New York, N. Y., 1945, p. 178.
- (8) Kutscher, W., *Z. Physiol. Chem.*, **283**, 268(1948).
- (9) Williams, R. T., "Detoxication Mechanisms," John Wiley & Sons, Inc., New York, N. Y., 1959, pp. 79, 80.