Quantitative Recovery of Radioactivity from \(^{14}\)C-Pentaerythritol Tetranitrate Administered to Rats

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A previous study (1) examined the absorption and subsequent metabolism of \(^{14}\)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 PET have been found (2-5). Although no evidence of expired \(^{14}\)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, \(^{14}\)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 \(^{14}\)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.—\(^{14}\)C-PE labeled at C-1 and C-2 was synthesized from acetaldehyde with specific activity of 1.8 mc./mmole and was employed to prepare \(^{14}\)C-PETN, m.p. 140-141.5\(^\circ\). 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.

\(^{14}\)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. \(^{14}\)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 Aerasite connected to a source of low-pressure air, followed by a gas scrubber containing water for humidifying 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 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 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 \(^{14}\)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 \(^{14}\)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.

Received April 22, 1966, from the Biochemistry Department, Warner-Lambert Research Institute, Morris Plains, N. J. 07950.

Accepted for publication June 8, 1966.
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 radioisochromatography 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 14C 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 quantitatively. The liver extract proved too intractable to produce a satisfactory chromatogram.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extract %</th>
<th>Residue %</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.61</td>
<td>0.02</td>
<td>0.63</td>
</tr>
<tr>
<td>Liver</td>
<td>0.24</td>
<td>0.08</td>
<td>0.32</td>
</tr>
<tr>
<td>GIT</td>
<td>54.73</td>
<td>3.92</td>
<td>58.65</td>
</tr>
<tr>
<td>Urine</td>
<td>25.21</td>
<td>—</td>
<td>25.21</td>
</tr>
<tr>
<td>Carcass</td>
<td>15.73</td>
<td>1.20</td>
<td>16.93</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.28</td>
<td>0.02</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>96.8</td>
<td>5.2</td>
<td>102.0</td>
</tr>
</tbody>
</table>

* Value from "5.5" rats.

The small amounts of radioactivity found in the tissues from the tissue extraction confirm the efficacy of the extraction procedure. The one notable exception is the liver extraction which removed only 7.5% 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 14CO₂ to the extent of only 0.28% of the 14C-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 (2). One possible source of the 14CO₂ 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 Krautz (7) indicated that some microorganisms degrade erythritol. Erythritol, of course, contains hydroxy 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

(6) Benedict, F. G., and MacLeod, G. J., Nutr., 1, 343 (1929).