METABOLISM OF GLYCERYL TRINITRATE TO CARBON DIOXIDE BY RATS

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Received May 1, 1968

Earlier reports indicate that glyceryl trinitrate undergoes only partial de-esterification in vivo (Needleman and Krantz, 1965; Needleman and Hunter, 1965) as well as in vitro (Oberst and Snyder, 1948; Heppel and Hilmoe, 1950; Hunter and Ford, 1955; Needleman and Krantz, 1965; Needleman and Hunter, 1965). We considered this limited metabolism to merit re-evaluation in view of the observations that pentaerythritol tetranitrate was degraded to pentaerythritol in mice (DiCarlo et al., 1965), rats (DiCarlo et al., 1967) and humans (DiCarlo et al., 1966). Since [1-^{14}C]- and [2-^{14}C]- glycerol were found to be converted extensively to $^{14}CO_2$ by the rat (Doerschuk, 1951; Gidez and Karnovsky, 1954), monitoring exhaled carbon dioxide for radioactivity was a direct method of determining whether ^{14}C-glyceryl trinitrate was converted to glycerol. After finding $^{14}CO_2$ to be produced from tagged glyceryl trinitrate, a comparative study was made of the rates of its elimination by rats administered [1,3-^{14}C]-glycerol and by rats administered [1,3-^{14}C]-glyceryl trinitrate.

MATERIALS AND METHODS

^{14}C-Glycerol - An aqueous solution of [1,3-^{14}C]-glycerol, purchased from New England Nuclear Corp., was diluted to 0.406 mg

of glycerol/ml and a specific activity of 4.88 μc/ml. The radiochemical purity was determined as 98.2%, 100% and 98.5% by thin layer chromatography in three solvents systems.

^{14}C-Glyceryl trinitrate - The glyceryl trinitrate used in this study was synthesized from [1,3-^{14}C]-glycerol by the nitration procedure of Lawrie (1928). Purification according to Dunstan et al.(1965) yielded a product with 99.9% radiochemical purity and a specific activity of 0.183 mc/g. In order to minimize the danger of explosion, the product was diluted with 19 parts by weight of C.P. lactose.

Animals - The animals used were female Wistar rats (Manor Farms) weighing 195-205 g.

$^{14}CO_2$ Collection - A syringe was used to administer 2 ml of an aqueous solution of [1,3-^{14}C]-glycerol (4.06 mg/kg) by gavage to three rats. Three 1 ml volumes of water were used to transfer residual ^{14}C-glycerol to the animal. Then the syringe was rinsed with water and the rinsings were counted for ^{14}C to calculate the dose actually administered. The animals were housed separately in glass metabolic units (Aerospace Industries) without food or water. The units were arranged so that all of the combined exhaled gases could be drawn by vacuum through either of two trains of eight gas washing bottles. The gases were passed through one train of scrubbers for 1 hr and then directed through the fresh train for an hr while the first series of bottles was emptied, washed, refilled with alkali and replaced. In this way respiratory carbon dioxide was collected without interruption from the same three rats. The collection periods were 1, 2, 4, 6, 8, 12, 18 and 24 hr. Each gas washing bottle contained 100 ml of aqueous alkali: 5% NaOH for hours 1 and 2, 10% NaOH for hours 4, 6 and 8, and 20% NaOH for hours 12, 18,

and 24. The contents of the washing bottles for each interval were combined and diluted to 2.0 l. for $^{14}CO_2$ assay.

The second experiment was conducted in the same manner with [1,3-^{14}C]-glyceryl trinitrate (10 mg/kg). The doses of glycerol and glyceryl trinitrate were equivalent (44 μmoles/kg).

$^{14}CO_2$ Assay - The $^{14}CO_2$ assay was carried out by gaseous diffusion of the carbon dioxide from acidified NaOH solution into hyamine hydroxide which was subsequently dissolved in scintillation fluid for counting. Two aliquots (1.0 and 2.0 ml) were taken from the NaOH solution representing a given collection period. Each aliquot was placed into a 25 ml erlenmeyer flask and closed with a rubber septum (Kontes) fitted with a polyethylene center well (Kontes) containing 0.2 ml of a 1 M solution of hyamine hydroxide in methanol (Packard). The NaOH solution in the flask was acidified by injecting 1.0 ml of 6 N H_2SO_4 through the septum with a hypodermic syringe. After 18 hr the center well was removed from the flask and placed into a vial containing 18 ml of scintillation fluid for counting.

RESULTS AND DISCUSSION

The data are plotted in Fig. 1 in terms of the percent of administered radioactivity converted to respiratory $^{14}CO_2$ with time. It is apparent that glyceryl trinitrate is absorbed very rapidly and that carbon dioxide is one of its major end products. A comparison of the rates of $^{14}CO_2$ elimination shows a 2 hr lag phase from glyceryl trinitrate which was not detected from glycerol.

Presumably, the trinitrate was de-esterified completely before oxidation. Judging from the failure of the drug to yield as much carbon dioxide as glycerol (36.5% vs. 51.8% in 24 hours) and the solubility of the drug metabolites, it seems probable

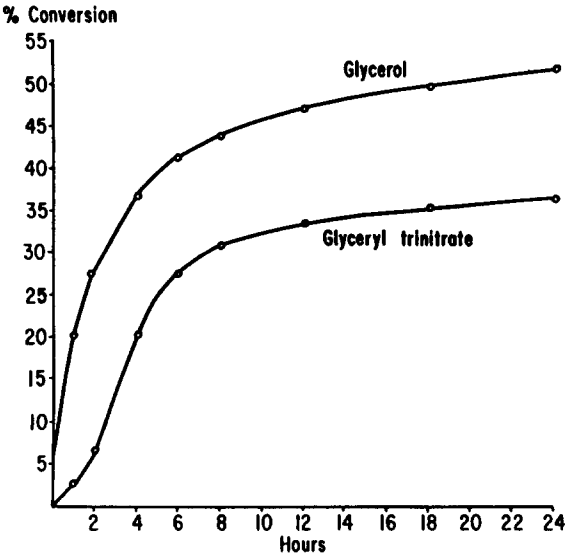

Fig. 1. $^{14}CO_2$ exhalation by rats following the oral administration of equivalent doses of [1,3-^{14}C]-glycerol and [1,3-^{14}C]-glyceryl trinitrate. % conversion refers to the percent of the administered ^{14}C converted to $^{14}CO_2$.

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that some of the metabolites were excreted into the urine. This matter is being investigated in order to assess the validity of the earlier reports (Needleman and Krantz, 1965; Needleman and Hunter, 1965) that the major urinary metabolites are glyceryl-1,2-dinitrate and glyceryl-1,3-dinitrate, and that no glycerol is passed into the rat urine. Obviously, the difficulty with the earlier studies was due to the use of non-radioactive glyceryl trinitrate.

To our knowledge, the present report is the third published study of $^{14}CO_2$ exhalation by rats dosed with ^{14}C-glycerol. Gidez and Karnovsky (1954) found that the position of the label, the dose and the route of administration (intragastric, intravenous, intraperitoneal) and no effect upon respiratory $^{14}CO_2$ production. In view of the fact that no two of the three $^{14}CO_2$

elimination curves are even approximately superimposable, we suggest that the nutritional state of the rat may be the determining factor. Doerschuk (1951) fasted his rats neither before nor during the study, and obtained the most complete conversion to $^{14}CO_2$. Gidez and Karnovsky (1954) fasted the animals for 24 hours before glycerol administration as well as during their six-hour study and noted the slowest $^{14}CO_2$ elimination. Our animals were fasted only during the 24-hour study period, and yielded a roughly intermediate $^{14}CO_2$ production curve. All three studies were conducted with Wistar strain rats; females were used only in the present investigation. In connection with oxidative metabolism, we offer the additional comment that the intestinal flora may induce significant variability.

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