Blood Nitrite and Nitrate Concentration After Oral and Intravenous Administration of Glyceryl Trinitrate in Rabbits

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Blood nitrite and nitrate concentrations were measured after oral and intravenous administration of glyceryl trinitrate at 1.0 mg./Kg. in rabbits. Nitrate and nitrite blood concentrations attained peak concentrations in 90–120 min. after oral administration and slowly returned to control levels. Nitrate and nitrite blood concentrations decreased slowly after intravenous administration of glyceryl trinitrate, then fell rapidly after 60 min. Blood nitrate concentrations were twice the blood nitrite concentrations 60 min. after either oral or intravenous administration of glyceryl trinitrate.

THE ABSORPTION of glyceryl trinitrate from the gastrointestinal tract has been questioned for over a half century. Most investigators believe the absorption to be poor on the basis of resulting physiological effects observed. Sollman states that when glyceryl trinitrate is ingested it is absorbed into the portal circulation and destroyed by the liver (1). Oral and intravenous administration of organic nitrates to dog and man have provided evidence that there is no correlation between blood nitrite and nitrate concentration and the observed physiological activity (2, 3). Recent investigations utilizing the observation of the vascular bed of the rabbit ear have shown that glyceryl trinitrate is absorbed in the gastrointestinal tract after oral administration (4).

The following study was undertaken to determine the blood nitrite and nitrate levels after oral and intravenous administration of glyceryl trinitrate in the rabbit.

METHODS

White New Zealand male rabbits weighing 3.5–4.5 Kg. were used in this study. The rabbits were lightly anesthetized with pentobarbital (25 mg./Kg., i.p.). One group of rabbits received glyceryl trinitrate orally (1.0 mg./Kg. from a stomach tube) in 1.5 ml. of water. A second group received glyceryl trinitrate intravenously (1.0 mg./Kg.) from a needle cannula in the marginal ear vein. The intravenous injection was made in 0.5 ml. of solution over a 60-sec. period. Blood samples were taken over a 2–3-hr. time period.

Each group of rabbits was used for only 3 successive blood samples, because of the large blood sample required (4 ml.) for the complete assay. A total of 30 rabbits was used for the assay. Blood samples were taken from an indwelling catheter (PE50) in the femoral artery.

Each 4-ml. blood sample was laked with 4 ml. of deionized water in a 125-ml. conical flask, and the following were added: 10 ml. of 0.2% solution of sulfanilamide, 2 ml. of 50% concentrated hydrochloric acid solution, and 2 ml. of 5% aqueous solution, prepared from mercuric chloride that had been sublimed 3 times. The resulting brownish mixture was thoroughly mixed by swirling and then centrifuged for 15 min. at 6000 r.p.m. in a horizontal centrifuge. The clear, deproteinated, decanted filtrate was quantitatively divided into two 10-ml. portions. One portion was used for the nitrite assay and the other for the nitrate assay (5).

Nitrite Assay.—To the 10-ml. portion of the deproteinated filtrate was added 1.0 ml. of 0.1% aqueous solution of *N*-(1-naphthyl)-ethylene diamine dihydrochloride to act as a coupling agent. Then the solution was allowed to stand for 10 min. and the color read on a Beckman DU spectrophotometer at the 540-mμ wavelength. The nitrite concentration was read from a previously determined standard curve prepared by plotting absorbance *versus* known concentrations of nitrite.

Nitrate Assay.—The remaining 10 ml. of the deproteinated filtrate was transferred to a 25-ml. test tube and hydrogen sulfide was bubbled through to precipitate unreacted mercury ions as mercuric sulfide. The excess hydrogen sulfide was removed by boiling the solution carefully to avoid bumping and subsequent loss of the solution. When all trace odor of hydrogen sulfide was removed, the murky solution was transferred to a 75-ml. conical flask, and 5.0 Gm. of Amberlite IR 120 AA (Mallinckrodt No. 3323), a strongly acidic, sulfonic cation exchange resin, was added.

The mixture was swirled for several minutes and centrifuged at 4000 r.p.m. for 10 min., or until all nitrite was absorbed, which was confirmed by placing 1 drop of coupler upon 1 drop of the decanted filtrate on a spot plate and by noting the absence of a color change. To the nitrite-free filtrate contained in a 125-ml. conical flask was added 5.0 ml. of 12% concentrated ammonium hydroxide solution and 1.0 ml. of 1% manganous chloride tetrahydrate to catalyze the reduction of nitrate ions. The solution was immediately placed in a water–ice bath maintained at 14 to 15° for 10 min. A 0.2 Gm. portion of zinc dust was added and the mixture stirred on a magnetic stirrer for 10 min. The slurry was filtered through a 3-in. funnel. To 5 ml. of the collected filtrate was added 1.0 ml. of *N*-(1-naphthyl)-ethylenediamine dihydrochloride, and the resulting solution was allowed to stand for 10 min., after which the absorbance was read at the 540-mμ wavelength. The nitrate concentration was read from a previously determined standard curve prepared by plotting absorbance *versus* known concentration of nitrate.

RESULTS

The results are presented in Fig. 1. By plotting the blood nitrate concentration and the blood nitrite concentration after intravenous injection, *versus* time, 2 curves running similarly were obtained. On the other hand, after oral administration the

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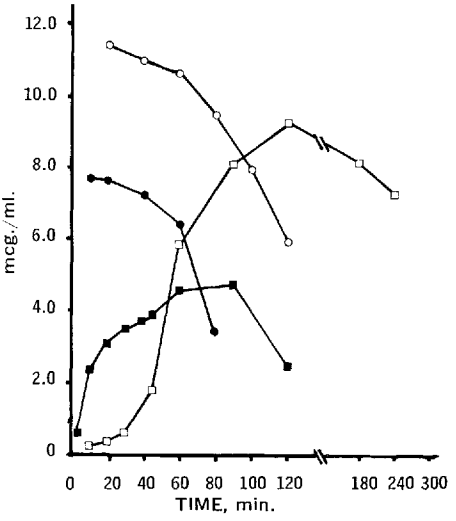

Fig. 1.—Micrograms of nitrate and nitrite per milliliter of blood in rabbits after oral and intravenous administration of glyceryl trinitrate, 1.0 mg./Kg. Key: O—O, nitrate, intravenous; ●—●, nitrite, intravenous; □—□, nitrate, oral; ■—■, nitrite, oral. Control blood nitrate level, 1.05 mcg./ml. ±0.54, as determined in 8 rabbits; control blood nitrite level, less than 0.50 mcg./ml., as determined in 6 rabbits.

initial nitrite concentration was higher than the nitrate concentration but was followed by a higher nitrate-to-nitrite concentration ratio of approximately 2:1 at 90 min.

DISCUSSION

The fact that blood nitrate and nitrite concentrations do not correlate with the time of peak effect of vasodilative action is well documented for glyceryl trinitrate and other organic esters of nitric acid (6). It is interesting to observe the prolonged high blood concentration of inorganic nitrate following either the intravenous or oral route of administration. Although the observed dilatation reported in the literature is said to last 60 min., these investigations show the blood concentration to remain significantly elevated 2–3 hr. after ingestion of glyceryl trinitrate at the dose reported. The possibility of buccal absorption is eliminated since the glyceryl trinitrate was placed directly in the stomach by intubation. Recently, DiCarlo, Hartigan, and Phillips have shown quantitative absorption of pentaerythritol tetranitrate by the gastrointestinal route (7). Crandall reported the enzymatic degradation of glyceryl trinitrate and related organic esters by erythrocytes in dogs (8). This enzymatic degradation was further correlated to the action of glyceryl trinitrate reductase in hog liver and heart (9). A similar enzyme system has been reported for pentaerythritol tetranitrate in human erythrocytes (10).

SUMMARY

Data for the blood nitrate and nitrite concentration after oral and intravenous administration of glyceryl trinitrate have been presented. After oral glyceryl trinitrate, the peak blood concentrations of nitrate were approximately twice that of nitrite. The blood nitrate and nitrite concentrations decreased similarly after intravenous administration of glyceryl trinitrate.

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