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EXTENSION OF THE BLOOD HALF-LIFE OF GLYCERYL TRINITRATE

INHIBITION OF GLUTATHIONE ORGANIC NITRATE ESTER REDUCTASE ACTIVITY IN THE RAT AND GUINEA PIG*

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Abstract—Cephalothin, penicillin G and probenecid inhibited GSH organic nitrate ester reductase (ONER) and several other enzymatic activities of GSH-*S*-transferases (EC 2.5.1.18) from rat and guinea pig liver. Erythrityl tetranitrate, a substrate for ONER, inhibited the aryl and alkyl transferase activities of two guinea pig liver GSH-*S*-transferases. These findings support the concept that ONER is one of the several activities possessed by the GSH-*S*-transferases. In an examination of possible *in vivo* action, parenteral administration of these inhibitors 2–30 min prior to i.v. administration of [^{14}C]glyceryl trinitrate resulted in a 50–100 per cent increase in the half-time of the metabolism phase of [^{14}C]glyceryl trinitrate clearance from the blood and postponed the appearance of metabolites. This presumably occurs through the *in vivo* inhibition of GSH–ONER activity of the GSH-*S*-transferases and suggests a possible means of prolonging the pharmacologic action of nitrate esters.

It has been a century since the English physician William Murrell [1] first demonstrated the effectiveness of sublingually administered nitroglycerin in the relief of angina pectoris. In general, many organic esters of nitric acid (especially polynitrates) reduce or prevent the angina and the hemodynamic manifestations of myocardial ischemia [2]. Although the precise mechanism by which organic nitrates exert their therapeutic effect is not clear, it most likely lies in their effectiveness as potent vasodilators [3].

A significant insight into the pharmacodynamics of these drugs occurred with the identification and partial purification from liver, by several different methods, of a GSH-dependent enzymatic activity which metabolizes organic nitrates [4–6], organic nitrate ester reductase (ONER).§ The overall reaction occurs according to equation 1.

$$R{-}O{-}NO_2 + 2\,GSH \xrightarrow{ONER} ROH + GSSG + NO_2^- + H^+. \quad (1)$$

Evidence exists [7, 8] that ONER is one of several activities possessed by the multi-functional enzymes, GSH-*S*-transferases (EC 2.5.1.18). These enzymes catalyze several reactions in which the sulfhydryl moiety of GSH attacks some electrophilic center, either displacing a leaving group or adding to alkenes or epoxides. In addition, these proteins bind a variety of chemically diverse compounds and have been implicated as being important in the transport of bilirubin in liver [9, 10] and of organic acids in kidney [11].

The metabolism of nitrate esters is quite rapid, possibly requiring only a single pass through the liver [12,13]. Thus, the development of long-acting nitrate ester preparations, either through the synthesis of a unique drug or the manufacture of a more effective pharmaceutical formulation, has been pursued [2, 14]. Some agents have been reported to possess the properties of a long-acting organic nitrate [14, 15]. However, the reports documenting the effectiveness of these drugs have been the subject of controversy [16, 17].

One approach to extending the pharmacologic lifetime of nitrate esters which has not yet been tried is to inhibit the enzymes which metabolize these drugs. Experiments are presented in this paper which increase the evidence that the GSH-*S*-transferases do have ONER activity and that inhibitors of the transferases will also inhibit the *in vivo* metabolism of organic nitrate esters.

METHODS

Partial purification of GSH-S-transferases from female guinea pig liver. Partial purification of these enzymes was performed according to the procedure of Hunter and O'Brien [18] at 0–4°. Briefly, saline

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§ Abbreviations: glutathione-organic nitrate ester reductase, ONER (EC 2.5.1.18); glyceryl trinitrate, GTN; erythrityl tetranitrate, ETN; reduced glutathione, GSH; oxidized glutathione, GSSG; 1-chloro-2, 4-dinitrobenzene, CDNB; 1, 2-dichloro-4-nitrobenzene, DCNB; and bovine serum albumin, BSA.

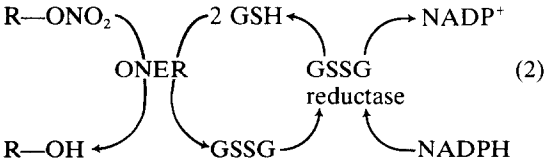
perfused guinea pig livers were homogenized in a solution containing 75 mM sucrose, 225 mM mannitol, 1 mM EDTA and 1 mg/ml of BSA. Centrifugation at 73,000 *g* for 1 hr removed insoluble material. The supernatant fluid was then concentrated by Amicon (Lexington, MA) PM10 membrane ultrafiltration, and the concentrated solution subjected to gel filtration on a Sephadex G-150 column (Pharmacia, Piscataway, NJ) (5 × 100 cm; eluant: 50 mM Tris–100 mM potassium phosphate and 1 mM EDTA, pH 8.4). The fractions corresponding to the peak of activity were pooled and applied directly to a CM Bio-Gel A column (Biorad, Richmond, CA) (2.5 × 45 cm). The column was developed with a 0–100 mM KCl gradient in 500 ml of 10 mM potassium phosphate, pH 6.7. The peak of activity from the previous gel filtration step was thus fractioned into three major peaks: a peak of activity which eluted from 57 to 160 ml (peak c) and two peaks, not resolved to baseline, which emerged from 215 to 251 ml (peak a) and from 285 to 340 ml (peak aa), following the designations that Jakoby and coworkers [8, 19] introduced for the rat. The lower case letters are used for guinea pig enzymes because the chromatographic peaks are in the same area but the substrate specificities are not identical with those in the rat, which are designated by capital letters.

Partial purification of female rat liver GSH transferases. Livers were removed from Sprague–Dawley rats, perfused with isotonic saline, homogenized in the solution containing 75 mM sucrose, 225 mM mannitol, 1 mM EDTA and 1 mg/ml of BSA, and centrifuged at 73,000 *g* for 45 min. The supernatant fraction was treated batchwise with DEAE-Sephadex in 10 mM Tris–HCl, pH 6.5 (0.25 g dry gel/g of liver). The gel was removed by filtration and washed with 3 vol. of 10 mM Tris–HCl buffer pH 8.0. The filtrate was treated with 0.66 g/ml of ammonium sulfate and allowed to stand overnight. This mixture was then centrifuged at 13,500 *g* for 30 min. The pellet was dissolved in a small volume of potassium phosphate buffer (10 mM, pH 7.4) and diafiltered with the same buffer until the ammonium sulfate was below 80 mM. Since 25 μl aliquots were used in enzyme assays, the final concentration of ammonium sulfate in the assay was about 2 mM.

Assays. GSH-*S*-aryltransferase activity was determined according to a slight modification of the procedure of Jakoby and coworkers [19, 20]. This method is based on the increase in absorbance at 340 nm ($\Delta\varepsilon = 9.6\ mM^{-1}\ cm^{-1}$) as GSH (0.5 mM) displaces the chloride from the substrate 1-chloro-2, 4-dinitrobenzene (CDNB) (0.1 mM) and produces *S*-(2, 4-dinitrobenzene) glutathione. This reaction is carried out in a 1-ml cuvette containing 100 mM potassium phosphate buffer, pH 6.5. The reference cuvette contained buffer and the aryl substrate. In some kinetic and inhibitor studies, the aryl substrate was 1,2-dichloro-4-nitrobenzene (DCNB), the pH 7.5, and the $\Delta\varepsilon$ 8.5 $mM^{-1}\ cm^{-1}$ at 345 nm. This substrate is relatively specific for GSH-*S*-transferases a and c.

GSH-*S*-alkyltransferase activity was measured titrimetically with a Radiometer TTT1 titrator. The reaction vessel contained 3.0 ml of a pH 7.2, non-buffered solution of 10 mM CH_3I and 1.5 mM GSH. The reaction was initiated with the introduction of a 20-μl aliquot of the protein solution and was monitored by the rate at which 5 mM NaOH had to be added to maintain a pH of 7.2. Because of the phosphate buffer in the enzyme aliquot, the final titration mixture was very weakly buffered with 0.06 mM phosphate.

ONER activity was measured by following the disappearance of A_{340} in a coupled reaction with glutathione reductase as follows [5]:



(2)

$NO_2^- + H^+$

In a routine assay, the final volume was 1.0 ml and contained 0.1 M potassium phosphate buffer at pH 7.4, 1 mM EDTA, 0.5 mM GSH, 0.1 mM NADPH, 1.6 units of glutathione reductase and 1 mM GTN. The reaction was started by the addition of GTN in 25 μl ethanol.

Inhibition kinetics. Initial velocities were determined for the conditions indicated in Figs. 1 and 2. Inhibition constants were determined from secondary plots of double reciprocal plots [21] and by a computer program which fits experimental data points to a kinetic model. For competitive inhibition the kinetic model was:

$$V_\phi = \frac{V_{max}[A]}{K_m\left(1 + \frac{[I]}{K_i}\right) + [A]} \tag{3}$$

and for non-competitive inhibition the kinetic model was:

$$V_\phi = \frac{V_{max}[A]}{K_m\left(1 + \frac{[I]}{K_i}\right) + [A]\left(1 + \frac{[I]}{K_{ii}}\right)}. \tag{4}$$

One of the substrates (GSH) was held constant. [*A*] is the concentration of the other substrate.

Determination of blood levels of [^{14}C]*GTN and metabolites in rat.* [^{14}C]-1,3-GTN (0.3 μCi/mg) was administered through a jugular vein cannula to a heparinized (100 units), pentobarbital-anesthetized (50 mg/kg) rat. Carotid artery blood samples (0.3 ml) were withdrawn from a carotid artery cannula at predetermined times and injected into 5 ml of petroleum ether in 15 × 130 mm screw cap tubes and rapidly mixed (Vortex mixer, Scientific Products, St. Louis, MO). Blood samples were treated a total of three times with 5 ml portions of petroleum ether to extract quantitatively [^{14}C]GTN [12]. The less hydrophobic metabolites of GTN were recovered quantitatively by one extraction with 5 ml of absolute ethanol [12]. These extracts were added to scintil-

Table 1. *In vitro* inhibition of guinea pig liver GSH-*S*-transferases a and c by erythrityl tetranitrate*

GSH-*S*-transferase	DCNB† (mM)	GSH (mM)	V_o	ETN‡ (mM)	V_i	Approximate K_i (mM)
a	0.66	1.0	0.125	0.51	0.048	0.3
a	0.66	0.4	0.094	0.25	0.054	
	0.66	0.4	0.094	0.51	0.035	0.3
a		0.4	0.180	0.6		
c	0.61	1.6	0.071	0.35	0.028	
	0.61	1.6	0.071	0.51	0.021	0.3
c	0.61	5.0	0.077	0.2	0.045	
	0.61	5.0	0.077	0.52	0.025	0.3
c		0.4	0.089	0.6		

* V = initial rate in μmoles min^{-1}. V_o = rate with DCNB alone or with ETN alone. V_i = rate of DCNB removal with ETN also present. $K_m^{ETN} = \sim 100\ \mu M$, $K_m^{GSH} = 100\ \mu M$ and $K_m^{DCNB} = 2.5$ mM. The initial rates with ETN and DCNB are 0.5–1 per cent of the rate with CDNB, which is not specific for transferases a and c.

† DCNB = 1, 2-dichloro-4-nitrobenzene. Disappearance was measured by the increase of A_{345} in 100 mM phosphate buffer, pH 7.5.

‡ ETN = erythrityl tetranitrate. Disappearance was measured by following NADPH disappearance fluorometrically with glutathione reductase in a coupled enzymatic assay at pH 7.5 (Needleman and Hunter [5]).

lation vials, the solvent evaporated just to dryness, and the residue was dissolved in 10 ml of scintillation fluid [4 g Omnifluor (New England Nuclear, Boston, MA)/liter toluene]. Evaporation just to dryness in a dessicator under low vacuum proved to be best. Excessive rates of evaporation, high air stream flow, and prolongation of the time beyond the point of dryness result in major losses of [^{14}C]GTN by volatilization. *In vivo* inhibition studies were carried out with the rat receiving a dose of inhibitor (i.v. or i.p.) at a preset time before the [^{14}C]GTN administration.

RESULTS

Interrelationships of GSH-S-transferase activities and ONER activity. Purified GSH-*S*-transferase a and c peaks from guinea pig liver were found to show ONER activity with ETN as substrate, as has been reported for rat. When ETN was present with the aryl transferase substrate DCNB, ETN behaved as an inhibitor of the aryltransferase activity (Table 1). Preliminary kinetic studies indicate a mixed type of inhibition. When GSH was at concentrations of 0.4 to 5 mM and DCNB was varied, the approximate

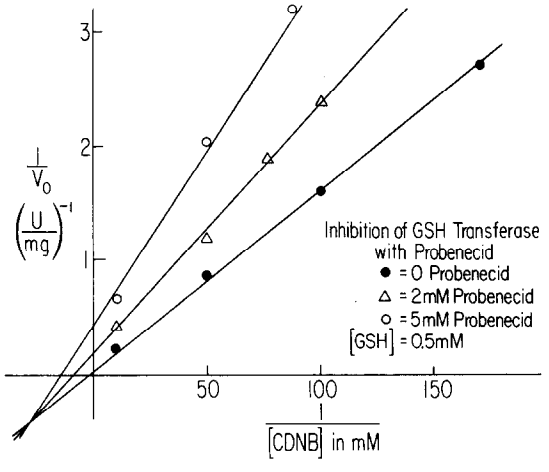


Fig. 1. Inhibition of guinea pig liver GSH-*S*-transferase c by probenecid. GSH was held constant and 1-chloro-2, 4-dinitrobenzene (CDNB) was varied. Lines were drawn by using the kinetic parameters calculated by fitting the data to a non-competitive inhibition model [22]. Activity unit = 1 μmole min^{-1} at 25°. $V_{max} = 18 \pm 1.3$ units/mg; $K_m^{CDNB} = 0.28 \pm 0.025$ mM; $K_i = 4.5 \pm 0.43$ mM; and $K_{ii} = 0.98 \pm 0.11$ mM.

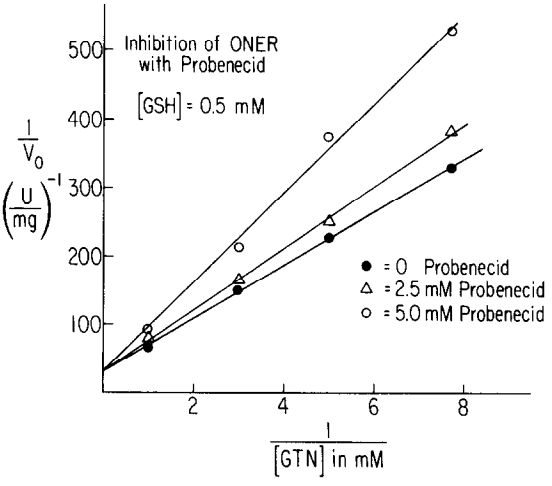


Fig. 2. Inhibition of guinea pig liver GSH-organic nitrate ester reductase activity of transferase c by probenecid. GSH was held constant and GTN was varied. Activity unit = 1 μmol min^{-1} at 25°. $V_{max} = 0.034$ units/mg: $K_m^{GTN} = 1.25$ mM; and $K_i^{probenecid} = 7.4$ for competitive inhibition.

Table 2. *In vitro* inhibition of partially purified rat liver GSH-*S*-transferase activities

Inhibitor	I_{50}* (mM)		
	ONER†	Aryltransferase‡	Alkyltransferase§
Probenecid	2.5	0.66	0.9
Penicillin G	15	5	0.5 mM (84% inhibition)
Cephalothin	2.2	0.61	

* Inhibitor concentration required for 50 per cent inhibition of activity. The number of data was too small for full kinetic plot and derivation of K_i.
† ONER = organic nitrate ester reductase. GTN = 1 mM, GSH = 0.5 mM.
‡ 1-Chloro-2,4-dinitrobenzene = 0.1 mM; GSH = 0.5 mM.
§ CH_3I = 10 mM, GSH = 1.5 mM.

K_i for ETN was 0.3 mM for transferase a and for c. When the other common aryl transferase substrate CDNB, which has a much higher affinity for the enzyme, was used, no inhibition of transferase a by ETN was observed.

Inhibition of GSH-S-transferase activities and ONER activity. Experiments were conducted to determine the sensitivity of ONER activity to inhibition by several substances [8, 10, 11] which bind to transferases and inhibit GSH-*S*-transferase activities. The substances chosen for initial studies, probencid, penicillin G and cephalothin, were selected from known transferase inhibitors because of low toxicity in animals and man. Probenecid inhibited both the aryl transferase and the ONER activities of purified guinea pig liver GSH-*S*-transferase peak c (Figs. 1 and 2). It appeared to be competitive with respect to GTN (Fig. 2), but showed hyperbolic mixed inhibition when the transferase substrate CDNB was studied (Fig. 1). This implies that the binding of probenicid to a second, non-competitive site has no effect on the GTN reaction but does affect the CDNB reaction [21, 22].

Probenecid, penicillin G and cephalothin also inhibited the alkyl transferase, aryl transferase and ONER activities of a partially purified preparation containing the mixture of rat liver GSH-*S*-transferases (Table 2). These inhibitor studies were carried out with the high affinity, very high rate of conversion substrate, CDNB.

Blood levels of [^{14}C]GTN and metabolites in rat. Studies were undertaken to determine if cephalothin and probenecid would retard the metabolism of [^{14}C]GTN *in vivo* in the rat.

Table 3 shows the results of control experiments in which the levels of [^{14}C]GTN and metabolites were followed in the blood of rats which had received no other drug. Figure 3 presents a semilog plot of a specific example of the blood levels of [^{14}C]GTN and metabolites vs time. There was a very rapid initial phase disappearance of [^{14}C]GTN ($T_{1/2}$ = 8 sec) as distribution to tissues occurred. This was followed by a slower but still rapid disappearance of GTN (metabolism phase, $T_{1/2}$ = 70 sec). The half-life for the metabolism of GTN agrees well with the value determined by Needleman and coworkers [12, 13]. The early rise of metabolite levels in the blood confirms that metabolism was occurring, and the metabolite level plateaued within 2 min (see also refs. 12, 13, 23).

Figures 4 and 5 illustrate [^{14}C]GTN blood clearance experiments in which cephalothin or probenecid was administered to rats prior to administration of [^{14}C]GTN. With both compounds a significant

Table 3. Summary of *in vivo* inhibition of organic nitrate ester reductase of the rat by probenecid and by cephalothin*

Drugs administered	$T_{1/2}$ for distribution phase† (sec)	$T_{1/2}$ for metabolism phase‡ (sec)
[^{14}C]GTN	8–11	70 ± 1.6 (5)
[^{14}C]GTN + cephalothin	10–16	98 ± 1.7 (3)
[^{14}C]-GTN + probenecid	13–32	171 ± 9 (6)

* Values are means ± standard error. The numbers in parentheses are the numbers of animals. Sprague–Dawley rats (230–317 g) were used. [^{14}C]GTN: 2.6 mg/kg, i.v., sp. act. 0.3 μCi/mg. Probenecid: 200 mg/kg, i.p., 30 min before [^{14}C]GTN, i.v. Cephalothin: 166 mg/kg, i.v., 10 min before [^{14}C]GTN, i.v.
† $T_{1/2}$ for rapid initial fall of blood level of [^{14}C]GTN, primarily distribution.
‡ $T_{1/2}$ for second slower phase of [^{14}C]GTN disappearance, primarily metabolism.

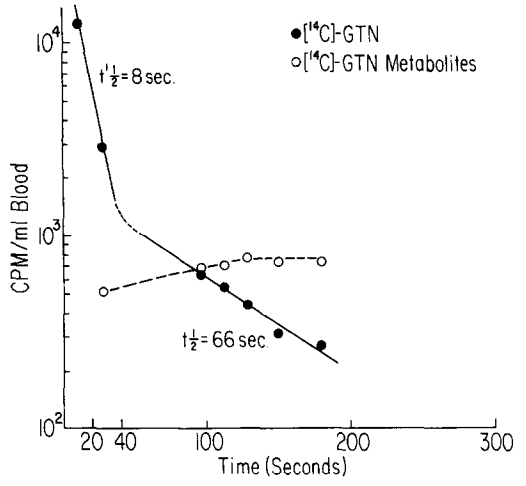


Fig. 3. Disappearance of [^{14}C]glyceryl trinitrate from, and appearance of ^{14}C-labeled metabolites in, the blood of the rat. Illustrative experiment. Use of a logarithmic scale for the ordinate permits illustration of the first order nature of the process responsible for disappearance of GTN. The appearance of ^{14}C-labeled products from the GTN is plotted on the same scale. The rate of product production appears proportional to the blood level of [^{14}C]GTN, which equilibrated rapidly with the liver cells. Metabolites appeared very early and continued to accumulate until the [^{14}C]GTN in the blood fell to low levels. The much slower removal of metabolites [12, 13] accounts for the maintenance of a plateau after blood [^{14}C]GTN had fallen to very low levels.

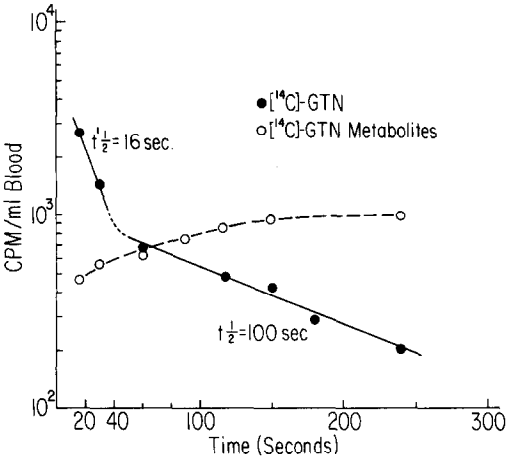


Fig. 4. Effect of cephalothin on [^{14}C]glyceryl trinitrate disappearance and formation of ^{14}C-labeled products. Illustrative experiment. A dose of 400 mg cephalothin/kg increased the $T_{1/2}$ for metabolism to 100 sec. The time for injection, mixing, distribution and withdrawing the first samples was so short that it was experimentally impossible to determine the $T_{1/2}$ for distribution with enough precision to draw any conclusion about possible changes.

increase in the half-time for [^{14}C]GTN metabolism was observed. In one experiment, the dose of cephalothin was more than doubled with no further increase in the observed half-time for metabolism. No conclusion can be drawn with respect to possible change in the $T_{1/2}$ for the initial distribution phase because of the great difficulty in getting meaningful values in the first 10–15 sec. Metabolites were always detected at the earliest time intervals and the level increased until the GTN level in the blood fell to about 50 per cent of the metabolite level.

Table 3 summarizes experiments with cephalothin and probenecid as *in vivo* inhibitors.

DISCUSSION

Extension of the pharmacologic lifetime of a drug has sometimes been realized through inhibition of its metabolism or elimination. A classic example is the inhibition by probenecid of the renal excretion of penicillin. Since the enzymatic activity which metabolizes organic nitrate esters has been identified and partially characterized as one of several activities possessed by many GSH-*S*-transferases, inhibitors of these enzymes offer the potential for slowing the metabolism of organic nitrate esters, i.e. inhibitors of transferase activities should inhibit the reductive hydrolysis of organic nitrate esters.

The inhibition by ETN of the aryl transferase (DCNB) activity of guinea pig liver GSH-*S*-transferases a and c supports the hypothesis that ONER activity is a transferase activity. Furthermore, in elution profiles for guinea pig liver extract from a CM-Biogel A column, transferase activity with CDNB and DCNB, and ONER activity with ETN show similar relative activity in the major GSH-*S*-transferase peaks.*

Probenecid is an inhibitor of GSH-*S*-transferase from human liver (K_i = 2.7 mM [10]). Cephalothin is a competitive inhibitor of rat liver GSH-*S*-transferases AA and B (K_i = 0.44 mM and 1.5 mM,

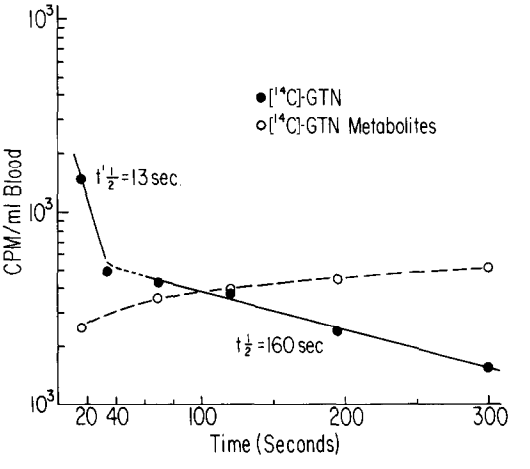


Fig. 5. Effects of probenecid on [^{14}C]glyceryl trinitrate disappearance and formation of ^{14}C-labeled products. Illustrative experiment. A dose of 200 mg probenecid/kg increased the $T_{1/2}$ for metabolism to 160 sec. The data in Table 3 suggest that on the average there might have been an increase of a few seconds in the $T_{1/2}$ for distribution, but the technical problems make a firm conclusion unwarranted.

* Unpublished data of Carl Irwin, Peter Chu and F. E. Hunter, Jr.

respectively) and a non-competitive inhibitor of rat liver transferases A and C ($K_i = 0.2$ mM for both) [8, 20]. These two drugs, as well as penicillin G, were tested as inhibitors of aryl- and alkyltransferase and ONER activities. Inhibition of all three activities was observed (Figs. 1 and 2 for guinea pig, Table 2 for rat).

The mechanism by which these compounds exert their inhibitory effects on the transferases is interesting. Transferase B (rat ligandin) has been shown to contain two binding sites: an active site in which catalysis of the transferase reaction occurs and a binding site to which many non-substrates bind and some substrates (e.g. bromosulfophthalein and benzyl chloride) bind [24]. Thus, the observation of both competitive inhibition and non-competitive or mixed inhibition by several inhibitors may reflect binding of the inhibitor to both the active site and the second binding site.

The *in vivo* experiments were undertaken to determine whether the transferase inhibitors could extend the plasma half-life of GTN in rat. Table 3 and Figs. 4 and 5 clearly show that probenecid or cephalothin administered to a rat prior to [^{14}C]GTN slowed the metabolism of the nitrate ester, as judged by the disappearance of [^{14}C]GTN from the blood and by the appearance of ^{14}C-labeled metabolites. The clearance of [^{14}C]GTN from blood was biphasic, as reported by Needleman and coworkers [12, 13]. The first phase was an exponential distribution from blood to tissues. This was so rapid that precise values are difficult to determine. The straight line in semilog plots of the second or metabolism phase of [^{14}C]GTN clearance from blood indicates that it also is exponential. Thus,

$$\frac{d[GTN]}{dt} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t}. \quad (5)$$

A_1 and A_2 are constants, and k_1 and k_2 are first order rate constants for distribution and for metabolism.

Inhibition of the second phase of [^{14}C]GTN clearance from the blood strongly indicates that the second phase is metabolism, a concept already supported by the early appearance of metabolites. Since this metabolism is first order, the concentration of GTN in the tissue is far below the K_m of the enzyme. All of the transferases showing ONER activity may not be inhibited by a single drug. Even with multiple transferase inhibitors, 100 per cent inhibition of metabolism might not be achieved due to an ONER activity that is not transferase related.

The metabolism of GTN in the rat appears to occur primarily in the liver [12, 13]. However, the kidney, with intermediate enzyme levels [25] and a large blood flow, and intestinal mucosa, with a specific activity on GTN that is higher than that for liver [25] probably contribute to metabolism. There is some disagreement as to whether any metabolism occurs in the erythrocyte. Needleman and coworker [12, 13, 23] reported very slow disappearance of GTN in blood *in vitro*, but several investigators think that some metabolism may occur in erythrocytes [26]. There may be species differences in this regard [27]. The only GSH-transferase reported for the erythrocyte (human) has been stated not to act on GTN [28].

Termination of GTN action [23, 25–27] may depend on destruction in other tissues as well as the liver, intestinal mucosa and kidney. Inhibition of the enzymes involved offers the possibility of slowing destruction at all sites. The dose of probenecid and of cephalothin used in the *in vivo* experiments would yield a level of 0.6 to 0.9 mM if evenly distributed in the body water of the rat. While this concentration would cause only 25 per cent inhibition of the ONER activity studied *in vitro* with 1 mM GTN (for rat, K_i for probenecid = about 1.3 mM, K_m for GTN = about 1 mM [8]), the concentration of inhibitor in the liver and other tissues may well be higher. In addition, the GTN concentration *in vivo* is probably much lower, perhaps 20–100 μM. The 59 per cent inhibition of ONER, implied when probenecid increases the metabolic $T_{1/2}$ from 70 to 171 sec, would be approximated by *in vivo* concentrations of 1.6 mM probenecid and 50 μM GTN.

In conclusion, reduction of the rate at which GTN is metabolized in rat can be achieved through the administration of certain drugs. This reduction presumably occurs through the *in vivo* inhibition of ONER activity of the GSH-*S*-transferases. The application of this method for prolonging the plasma half-life of GTN is suggested as having potential therapeutic value for the treatment of ischemic heart disease and the relief of angina pectoris. Further studies are needed to find better inhibitors and to determine what physiologic disturbance may result from a high degree of *in vivo* inhibition of the GSH-*S*-transferases.

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