movement phenomena and it liberates and absorbs ions ($Na^+$, $K^+$, $Cl^-$, and $HCO_3^-$) to maintain in its environment an ionic composition similar to that of interstitial extracellular fluid. However, it is possible to perform studies under controlled pH conditions, if one compromises tonicity (tromethamine buffer, pH 7.95 ± 0.005, buffer capacity = 0.225). It also appears that studies at various pH's will be possible using the same system under postmortem conditions.

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# Enzyme Induction of Organic Nitrates I: Nitroglycerin *In Vivo* Experiments


**W. A. RITSCHEL▲, G. RITSCHEL-BEURLIN, and T. E. ELING**



**Abstract** □ Upon oral administration of nitroglycerin–proxyphylline tablets with timed release to humans, it was observed that the nitroglycerin blood levels declined after the 5th day. Experiments in rabbits and mice were performed to clarify whether this phenomenon is caused by enzyme induction. Oral administration of 0.1 and 0.2 mg./kg. nitroglycerin to rabbits caused a decrease in the peripheral maximal temperature rise. The decline in pharmacological action depends on dosage and apparently follows first-order kinetics. Pretreatment of mice with either nitroglycerin or pentobarbital caused a similar decrease in pentobarbital sleeping time, indicative of enzyme induction. However, in contrast to the hepatic response elicited by barbiturate pretreatment in which the liver weights increased, nitroglycerin caused a significant decrease, thereby suggesting a different mechanism of action for the vasodilator.



**Keyphrases** □ Enzyme induction of organic nitrates—nitroglycerin, rabbits, mice □ Nitroglycerin—enzyme induction, rabbits, mice □ Vasodilation, nitroglycerin—enzyme induction, rabbits, mice


Although nitroglycerin is well established in the therapy of angina pectoris, its oral absorption has long been questioned. Salter (1) stated that nitroglycerin is not decomposed by the gastric juice, but its rate of absorption is slow. Sollmann (2) mentioned that nitroglycerin is more potent when administered buccally instead of orally, because by the latter route nitroglycerin is absorbed into the portal circulation and then destroyed by the liver. That nitroglycerin is also absorbed—at least in the rabbit—when given orally was shown by Turner (3), Lorenzetti *et al.* (4), and Bogaert *et al.* (5). Ritschel and Clotten (6) proved oral absorption of nitroglycerin in humans, calculating (7) a biological availability of orally given nitroglycerin of 36 and 55% for doses of 0.8 and 1.6 mg., respectively, to adults compared to the blood levels upon buccal absorption.

Since nitroglycerin is especially valuable when used prophylactically (8, 9), several oral timed-release nitroglycerin preparations have been developed, clinically tested, and marketed (10–18). By using an oral timed-release proxyphylline preparation (19–22), nitroglycerin was incorporated into this formula and biopharmaceutically evaluated (6, 7); it was found that the nitrate plasma level decreased after the 5th day (Fig. 1). This observation led to the suspicion of a possible enzyme induction (23).

To study the possibility of enzyme induction (23), further experiments were performed using a pharmacological parameter other than plasma levels, because there seems to be no correlation between hypotensive

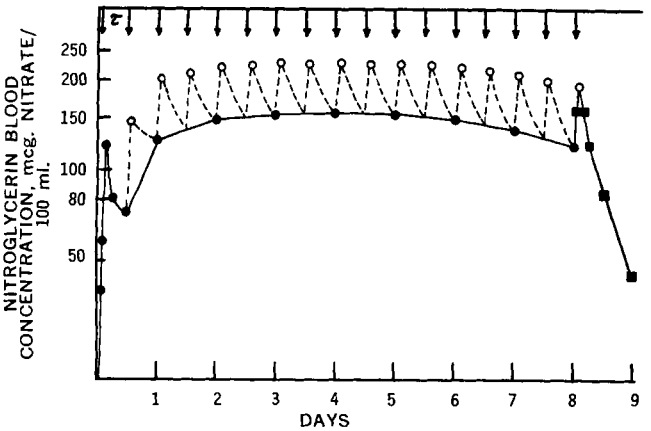


**Figure 1**—*Nitrate plasma level upon oral administration of a timed-release nitroglycerin (5.0 mg.)-proxyphylline (490 mg.) tablet (6, 7). Key: ↓, oral administration of one tablet of timed-release nitroglycerin; τ, dosing interval of 12 hr.; ●, $C'_{min.}$, minimum nitrate plasma concentration just before administration of the next dose; ■, additional blood levels determined; and ○, computed maximum nitrate plasma concentration $C'_{max.}$.*

action of certain aliphatic nitric acid esters and the amount of nitrate and nitrite ions in the plasma (24). Onset and action in small doses of organic nitrates are observed to be as rapid as inorganic nitrate, and the clinical effects do not correlate with an elevation of blood nitrite (25).

The effects of nitroglycerin on peripheral (noncoronary) vessels were of particular interest pharmacologically, because at least part of nitroglycerin's efficacy seems to be due to its effect on the peripheral vasculature (26). First the method described by Turner (3) was used in observing the dilatation of the vascular bed of the rabbit's ear, but it has not been very successful in quantifying vasodilatation. Therefore, rectal temperature measurement was then used, employing a thermo probe.

Enzyme induction was determined by using sleeping time in mice upon administration of barbiturates, which induce hepatic enzymes as described by Conney (27) and Remmer (28). The test for increase in liver weight, which has been observed upon enzyme induction with barbiturates (28), was used to determine whether nitroglycerin is metabolized by the same system as barbiturates.

## METHODS

**Vasodilatation**—Male, New Zealand, white rabbits, weighing

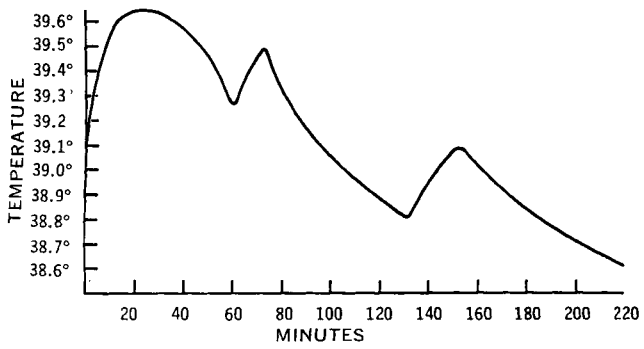


**Figure 2**—*Typical fluctuating temperature curve upon nitroglycerin administration, 0.1 mg./kg. orally, on 7th day of administration.*

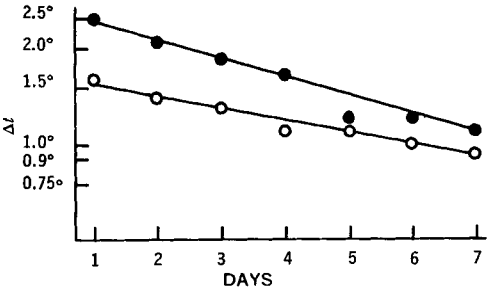


**Figure 3**—*Semilog plot of pharmacological decline of peripheral temperature. Key: ●, 0.2 mg./kg. nitroglycerin; and ○, 0.1 mg./kg. nitroglycerin.*

2.55–3.06 kg., were used in this study. The rabbits were maintained in the same room with pelletized standard food and water *ad libitum* and were assigned to two groups of three each. Both groups received nitroglycerin in aqueous solution orally by means of a stomach catheter (to avoid any buccal absorption), and the tube was rinsed after administration with 3 ml. distilled water. The administration was repeated at exactly 24-hr. intervals for 7 days. One group received a daily dose of 0.1 mg./kg.; the other received 0.2 mg./kg. After nitroglycerin administration the rabbits were kept in a restraining box and the rectal temperature was measured using a tele-thermometer[1] for a period of 2 hr., taking temperature readings at 2-min. intervals. The normal body temperature, as measured before the nitroglycerin administration, was subtracted from the readings and the result was recorded as $\Delta t$ in degrees Centigrade.

**Sleeping Time**—White, inbred, male Swiss Webster mice of the same age, with a body weight of 31.6–35.4 g. (mean body weight 32.91 g.), were maintained in the same room with pelletized standard food and water *ad libitum*. They were divided into four groups of 40 mice each. The control group received 50 mg./kg. sodium pentobarbital intraperitoneally. The second group was pretreated with carbon tetrachloride once daily for 5 days. For pretreatment the mice were put in a closed jar with a cotton pledget soaked in carbon tetrachloride until incoordination of movement was observed. Twenty-four hours after pretreatment, the mice got 50 mg./kg. sodium pentobarbital intraperitoneally. The third group was pretreated with 40 mg./kg. sodium pentobarbital intraperitoneally once a day for 5 days. Twenty-four hours after pretreatment, the mice were given 50 mg./kg. sodium pentobarbital intraperitoneally. The fourth group was pretreated with 0.2 mg./kg. nitroglycerin orally by use of a stomach catheter once a day for 5 days. Twenty-four hours after pretreatment, the mice received 50 mg./kg. sodium pentobarbital intraperitoneally. Onset of sleeping time (loss of righting reflex) and duration were determined in minutes.

**Liver Weight**—White, inbred, male Swiss Webster mice of the same age (40 days), with a body weight of 18.2–24.9 g. (mean body weight 21.6 g.), were divided into two groups of 20 mice each. The control group was given distilled water orally using a stomach catheter once a day for 10 days. The test group received 0.2 mg./kg. nitroglycerin in aqueous solution once a day by means of a stomach catheter for 10 days. Twenty-four hours after the last administration, the mice were sacrificed and their body and liver weights were taken.

## RESULTS

**Vasodilatation**—While recording temperature, fluctuations were observed which the authors attributed to redistribution phenomena from blood circulation into peripheral compartments and back into the central compartment. A typical temperature curve is shown in Fig. 2. The highest temperature differences, $\Delta t$, were plotted on semilog paper *versus* time in days. By assuming a first-order reaction for the decline in pharmacological effect, the rate constant $k_E'$ was calculated according to Eq. 1:

$$k_E' = \frac{\ln \Delta t_1 - \ln \Delta t_2}{d_2 - d_1} \qquad \text{(Eq. 1)}$$

[1] Model 43, probe No. 408, Yellow Springs Instrument Co.

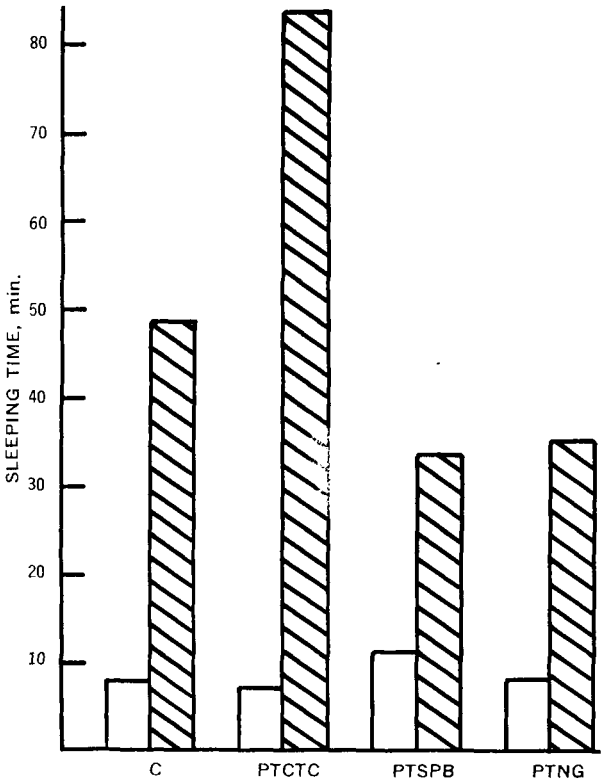


**Figure 4**—*Sleeping time in mice upon peroral administration of 50 mg./kg. sodium pentobarbital. Key: C, control; PTCTC, pretreatment with carbon tetrachloride; PTSPB, pretreatment with sodium pentobarbital; PTNG, pretreatment with nitroglycerin;* □, *onset; and* ▨, *duration.*

where $k_E'$ = apparent first-order rate constant for decline of pharmacological effect, ln $\Delta t_1$ and ln $\Delta t_2$ = ln of temperature differences in degrees Centigrade at two different times after administration of nitroglycerin orally, and $d_1$ and $d_2$ = time interval in days.

The semilog plot is given in Fig. 3. The decline in pharmacologic effect due to supposedly enzyme induction was found to be $k_E'$ = 0.126 [day$^{-1}$] for the dose of 0.2 mg./kg. and $k_E'$ = 0.080 [day$^{-1}$] for the dose of 0.1 mg./kg. nitroglycerin. The enzyme induction apparently depends on dosage (29).

**Sleeping Time**—The normal sleeping time of the control group after oral administration of sodium pentobarbital was 48.8 min. After treatment with carbon tetrachloride, which resulted in liver damage, the sleeping time after oral administration of sodium pentobarbital increased to 83.7 min. Upon pretreatment with sodium pentobarbital (enzyme inducer), the sleeping time decreased to 33.6 min. and with nitroglycerin to 35.0 min. Nitroglycerin seems to be an enzyme inducer for barbiturates. The results (mean of each group) are given in Fig. 4.

**Liver Weights**—Body weights increased during the time of the experiment from 26.8 to 26.9 g. in the control group and to 27.7 g. in the test group treated with nitroglycerin. The mean liver weight in the control group after the experiment was 1.61 g., and that of the test group was 1.43 g. Statistically analyzed, the liver weights of the test group were significantly lower (>99%) than in the control group. Nitroglycerin enzyme induction is apparently not associated with increased endoplasmic protein synthesis and differs in mechanism of enzyme induction from that of barbiturates.

## DISCUSSION

The results show that nitroglycerin orally administered by use of a stomach catheter is absorbed as well in rabbits as in mice, causing peripheral vasodilatation measured by a rise in temperature. The pharmacological effect of vasodilatation decreases with repeated doses. The decline apparently follows first-order kinetics and depends on dosage, *i.e.*, the higher the dose, the higher the rate of decline. The decrease in pharmacological response as observed in animals seems to have its parallel in humans, because a decrease in plasma levels has been found on multiple dosing and since it is clinically well known that nitroglycerin's efficacy decreases with continuing therapy. The anticipated explanation for that phenomenon is enzyme induction (23–29), as supported by the recent findings of Bogaert *et al.* (5) that on pretreatment with phenobarbital, nitroglycerin plasma levels were lower than in normal rabbits. Furthermore, there is a nitroglycerin–foreign enzyme induction in addition to the nitroglycerin–autoenzyme induction, because pretreatment with nitroglycerin results in the reduction of pentobarbital sleeping time. Yet, both enzyme-inducing substances, *i.e.*, nitroglycerin and barbiturates, have apparently different mechanisms of enzyme induction as shown by the liver weight test.

Further reports will deal with the microsomal study of enzyme induction of nitroglycerin and other organic nitrates, group enzyme induction of organic nitrates, mechanism of enzyme induction of nitroglycerin, and its clinical implications.

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