was a linear relationship with a slight increase in adhesiveness as the relative humidity was increased.

With Products A and C, the maximum value of adhesiveness occurred at ~30% RH. With Product A, a minimum value of adhesiveness was reached at 70% RH, and then the adhesiveness increased with a further increase in humidity. With Product C at >50% RH, the tablet and the coating became spongy and deformed so that measurements could not be made.

Since the coating material, solvent, coating process, and substrate were unknown, speculation on these differences serves no useful purpose; however, the results confirm the applicability of this method to the detection of changes occurring in a film-coated product.

Influence of Solvent—The solvent from which a film coating is applied may affect the adhesion of the film to the substrate (2). When hydroxypropylcellulose was applied from the five solvent systems shown in Table III, there was a twofold difference ($1.44–2.98 \times 10^4$ Nm^{-2}) in adhesiveness among the organic solvents. When hydroxypropylcellulose was applied from aqueous solution, the adhesiveness (0.71×10^4 Nm^{-2}) was one-fourth to one-half as great as that from the organic solvents.

Applications—The method may be used to evaluate the stability of a film-coated tablet to moisture and its physical stability during shipment. The method also may be used in product development to express quantitatively the bonding between the film coating and the tablet surface and to compare the effect of various solvents on the bonding.

REFERENCES

(1) J. A. Wood and S. W. Harder, *Can. J. Pharm. Sci.*, **5**, 18 (1970).

(2) P. D. Nadkarni, D. O. Kildsig, P. A. Kramer, and G. S. Banker, *J. Pharm. Sci.*, **64**, 1554 (1975).

(3) D. G. Fisher and R. C. Rowe, *J. Pharm. Pharmacol.*, **28**, 886 (1976).

(4) R. C. Rowe, *ibid.*, **29**, 58 (1977).

(5) *Ibid.*, **30**, 343 (1978).

(6) *Ibid.*, **29**, 723 (1977).

(7) A. Wexler and W. G. Brombacker, *Natl. Bur. Stand. (U.S.) Circ.*, **1951**, 512.

(8) F. Proschan, *Am. Stat. Assoc. J.*, **48**, 550 (1953).

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Role of α- and β-Adrenergic Activation in Ventricular Fibrillation Death of Corticoid-Pretreated Rats

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Received March 5, 1979, from the *Department of Pharmacology, New York Medical College, Valhalla, NY 10595.* Accepted for publication November 8, 1979.

Abstract □ Death in ventricular fibrillation was induced consistently in desoxycorticosterone acetate-pretreated rats by the β-adrenergic agonist isoproterenol but not by norepinephrine or epinephrine, both of which possess α- as well as β-adrenergic activity. Aminophylline, which enhances β-adrenergic activity, and phenoxybenzamine, an α-receptor blocking agent, were used to study the roles of α- and β-adrenergic stimulation in the production of ventricular fibrillation. With the addition of aminophylline, both norepinephrine and epinephrine produced death in ventricular fibrillation, and the existing cardiotoxicity of isoproterenol was potentiated. Similarly, in the presence of phenoxybenzamine, doses of norepinephrine and epinephrine that had been well tolerated became lethal. Interventions that favor β-adrenergic preponderance, either by enhancing β-effects or by blocking protective α-adrenergic activation, apparently increase the arrhythmogenic propensity of norepinephrine and epinephrine in steroid-pretreated rats. The similarity of some forms of stress to the experimental protocol of chronic steroid treatment followed by acute catecholamine exposure is discussed.

Keyphrases □ Ventricular fibrillation—role of α- and β-adrenergic activity, desoxycorticosterone acetate-pretreated rats □ Arrhythmias—catecholamine induced, effect of steroid pretreatment □ Aminophylline—effect on catecholamine-induced arrhythmias, steroid-pretreated rats □ Phenoxybenzamine—effect on catecholamine-induced arrhythmias, steroid-pretreated rats

Earlier studies in this laboratory showed that, following pretreatment of the albino rat with the steroid desoxycorticosterone acetate and saline as the drinking fluid, the administration of isoproterenol elicited severe cardiac arrhythmias at dose levels that otherwise are well tolerated (1, 2). The enhanced cardiotoxicity was reflected in a shift of the isoproterenol LD_{50} from 680 mg/kg (3) in untreated rats to 14.5 μg/kg in desoxycorticosterone acetate–saline-pretreated rats (4), a nearly 47,000-fold potentiation. The deaths produced by this drug–drug interaction usually occurred within 60 min and consistently were due to ventricular fibrillation, while the mortality observed in untreated rats developed over 24 hr and usually was attributable to acute heart failure, lung edema, and shock. Recent studies showed that prednisone, administered either subcutaneously or orally, also can sensitize the myocardium to the arrhythmogenic effect of isoproterenol (4). It has been suggested that this type of drug interaction may expose patients such as asthmatics, taking steroids and isoproterenol simultaneously, to potentially life-threatening cardiac arrhythmias (5).

The mechanism underlying this phenomenon is not well understood. Alterations in the myocardial electrolyte content may be a factor in the increased myocardial vulnerability to isoproterenol. Electrolyte changes such as those seen with this treatment (6) have been well documented as increasing the propensity of the heart to arrhythmias and ventricular fibrillation (7).

The ability of steroids to affect protein synthesis also might be important in the sensitization process. In preliminary studies, agents that inhibit protein synthesis blocked the steroid-induced myocardial sensitization to isoproterenol (8).

The present investigation was undertaken to determine: (*a*) whether norepinephrine and epinephrine, which are both α- and β-adrenergic agonists, exhibit enhanced arrhythmogenic activity following steroid pretreatment comparable to that observed with isoproterenol; and (*b*) whether aminophylline, an agent administered frequently

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Table I—Effect of Norepinephrine and Epinephrine on the Desoxycorticosterone Acetate–Saline-Pretreated Rat

Dose, μg/kg	l-Norepinephrine Bitartrate		l-Epinephrine Bitartrate	
	n	Mortality, %	n	Mortality, %
25	—	—	7	0
50	7	0	5	0
75	—	—	7	0
202	—	—	5	0
300	22	0	—	—
680	—	—	5	0
1360	—	—	5	0

to asthmatics alone or with steroids and catecholamines, affects the existing interaction between corticoids and adrenergic agonists.

EXPERIMENTAL

Male albino rats, 250–300 g, were pretreated with desoxycorticosterone acetate (I) and saline as the drinking fluid. Pellets of the steroid were implanted subcutaneously and provided a constant source of I over the entire 21-day pretreatment period. The methodology for the manufacture and implantation of these pellets was described previously (4). Except where noted, all animals received steroid pretreatment.

All drug solutions were prepared in normal saline immediately prior to injection, except for phenoxybenzamine, which was suspended in a 10% acacia solution. All drugs were administered subcutaneously. Aminophylline, 75 mg/kg, was administered to rats 10 min prior to the catecholamines; chlorisondamine, 10 mg/kg, and phenoxybenzamine, 4 mg/kg, were given 30 min before the catecholamines.

Animals were observed for 90 min following catecholamine administration, and mortality rates were noted. ECG's were monitored from selected animals in each group to observe arrhythmias and to verify that mortality was due to ventricular fibrillation. The ECG was obtained by taping gold-plated disk electrodes to each limb of the rat, which otherwise was unrestrained.

The data obtained are reported as mortality rates. This selection was made because mortality rates reflect both the relative toxicities of the various agents and their relative arrhythmogenic activity since early death was attributable consistently to ventricular fibrillation.

A χ-square analysis was used to determine significant differences between treatment groups. Linear regression was used to determine the best-fit lines through data points on the graphs and to calculate LD_{50} values.

RESULTS

Effect of Norepinephrine and Epinephrine—Several doses of norepinephrine and epinephrine were administered to I–saline-pretreated rats (Table I). Within the dose ranges used, there was no mortality. The ECG's recorded from some rats did not show any of the tachyarrhythmias usually seen with isoproterenol in I–saline-pretreated rats. The largest dose of epinephrine did elicit bradyarrhythmias, which often are seen with the substantial elevations of blood pressure produced by this dose of epinephrine. However, these bradyarrhythmias are unlike the arrhythmias produced by isoproterenol.

Table II—Toxicity of Aminophylline in the Desoxycorticosterone Acetate–Saline-Pretreated Rat

Treatment Group	n	Number of Deaths	Mortality, %
I Aminophylline (75 mg/kg)	9	7	77
II Aminophylline + handling	9	6	67
III Aminophylline + saline injection	17	14	82
IV Aminophylline + saline injection + chlorisondamine	15	1	7[a]
V No steroid; aminophylline + saline injection	10	0	0[a]

[a] $p < 0.001$ compared to Group III.

Table III—Effect of Aminophylline on Catecholamine Cardiotoxicity

	Dose, μg/kg	Aminophylline, I–Saline, and Chlorisondamine		LD_{50}, μg/kg	I–Saline and Chlorisondamine Mortality,	
		n	Mortality, %		n	%
l-Norepinephrine bitartrate	30	13	8			
	100	10	50	110		
	200	16	62			
	300	20	90		15	0[a]
l-Epinephrine bitartrate	10	11	18			
	30	10	60	28.2		
	100	8	87		10	0[a]
l-Isoproterenol bitartrate	2	10	20			
	3	9	44	3.63		
	10	10	90			

[a] $p < 0.001$ compared to the group with aminophylline.

Cardiotoxicity of Aminophylline—The administration of aminophylline, 75 mg/kg, to I–saline-pretreated rats resulted in a 77% incidence of mortality (Table II). Animals in Group II were handled 10 min after aminophylline administration and showed a 67% incidence of mortality. Group III animals were given an injection of normal saline (0.1 ml/100 g) 10 min after aminophylline administration, resulting in a mortality rate of 82%.

Although a direct effect of aminophylline on the heart could not be ruled out, past experience showed that β-adrenergic stimulation was necessary to produce these deaths in ventricular fibrillation. To determine whether reflex release of endogenous catecholamines in response to the stress of handling, the injections, or simply the laboratory environment was the cause of these deaths, the ganglionic blocking agent chlorisondamine, 10 mg/kg, was used. Animals in Group IV received this agent 30 min prior to aminophylline admininstration (Table II). Ganglionic blockage was expected to prevent reflex release of catecholamines and to help determine whether the observed toxic effect was attributable to a direct action of aminophylline on the heart.

Following chlorisondamine administration, the combination of aminophylline followed by a saline injection produced only a 7% mortality rate, a highly significant decrease from the 82% seen without chlorisondamine. This result was compatible with the assumption that stress-induced reflex release of catecholamines, and not a direct effect of aminophylline, was responsible for the observed high death rate.

The importance of steroid pretreatment to this response is illustrated by the data for Group V (Table II). These animals were given aminophylline, 75 mg/kg, followed by a saline injection, but they did not receive I–saline pretreatment. Their mortality rate was significantly different ($p < 0.001$) from that of Group III, which had received steroid pretreatment in addition to the aminophylline and saline injections.

Toxicity of Catecholamines following Aminophylline—In this study and all following studies, chlorisondamine was used routinely together with aminophylline to block endogenous catecholamine release in order to evaluate the effect of aminophylline on administered catecholamines.

Table III shows the effect of aminophylline on norepinephrine and epinephrine toxicity. Unlike the initial study in which administration of the two agonists produced no fatality, dose-dependent increases in the mortality rate were observed with both catecholamines. Data for isoproterenol have been included to show its relative potency. The calculated LD_{50} values show that isoproterenol was nearly seven times more potent than epinephrine, which, in turn, was about four times more active than norepinephrine. The role of aminophylline in eliciting this cardiotoxicity is seen when comparing the effect of equal catecholamine doses in its presence and absence. Table III shows that in the presence of aminophylline, chlorisondamine, and I–saline, norepinephrine, 300 μg/kg, and epinephrine, 100 μg/kg, produced mortality rates of 90 and 87%, respectively. Without aminophylline, neither drug produced any mortality at equal dose levels.

Unlike norepinephrine and epinephrine, isoproterenol produced ventricular fibrillation deaths without aminophylline, but its effect with aminophylline was enhanced greatly (Fig. 1). The isoproterenol used in this study was the racemic hydrochloride salt, not the l-isomer of the

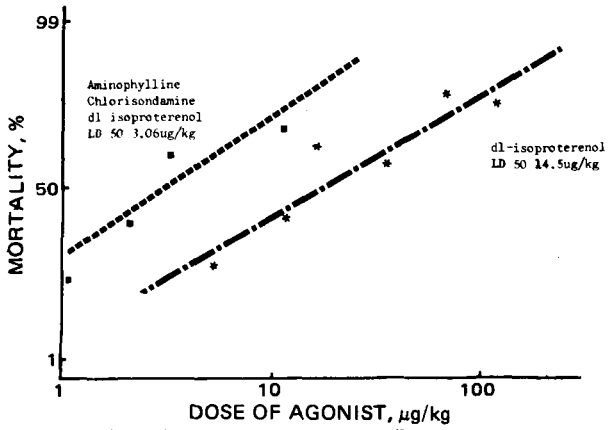

Figure 1—*Effect of aminophylline (75 mg/kg) on isoproterenol cardiotoxicity in desoxycorticosterone acetate-saline-pretreated rats after ganglionic blockage with chlorisondamine (10 mg/kg).*

bitartrate used in all other studies. The curve on the right represents isoproterenol toxicity following I–saline pretreatment alone. The addition of aminophylline (and chlorisondamine to prevent release of endogenous catecholamines) produced a marked shift to the left, decreasing the LD_{50} value from 14.5 to 3.06 μg/kg, a nearly fivefold potentiation (95% confidence limits of the potentiation were 2.48–9.4 times).

Effect of α-Blockage on Catecholamine Toxicity—While isoproterenol alone could produce ventricular fibrillation deaths in I–saline-pretreated rats, norepinephrine and epinephrine did so only in the presence of aminophylline. Phenoxybenzamine, an α-adrenergic blocking agent, was used to determine whether the potent α-agonist properties of norepinephrine and epinephrine were protecting the animals from fibrillation deaths (Fig. 2). Norepinephrine, 300 μg/kg, produced no deaths in I–saline-pretreated rats; but with the addition of the α-blocker, a 40% mortality rate was produced. Similarly, 100 μg of epinephrine/kg was nontoxic in steroid-pretreated rats; but in combination with phenoxybenzamine, it produced a 70% mortality rate.

Phenoxybenzamine itself did not show any acute toxicity in I–saline-pretreated rats. Ten animals injected with the drug followed by a saline injection did not exhibit any signs of the lethal interaction. Blockage of the α-adrenergic component of norepinephrine and epinephrine apparently allows the unopposed β-adrenergic activity to produce fibrillation deaths in these animals in a manner similar to that of the pure β-agonist isoproterenol.

Effect of Norepinephrine on Isoproterenol Cardiotoxicity—The interaction between α- and β-adrenergic stimulation was examined from a different perspective. In this study, a dose of *l*-isoproterenol, which produced a 75% mortality rate, was administered 10 min after 300 μg of norepinephrine/kg. As shown in Fig. 2, this dose of norepinephrine produced fibrillation deaths only if its α-adrenergic activity was blocked. Since the α-agonist component of norepinephrine prevented this catecholamine from inducing fibrillation deaths, it was expected that it also might attenuate the response of the pure β-agonist isoproterenol. This

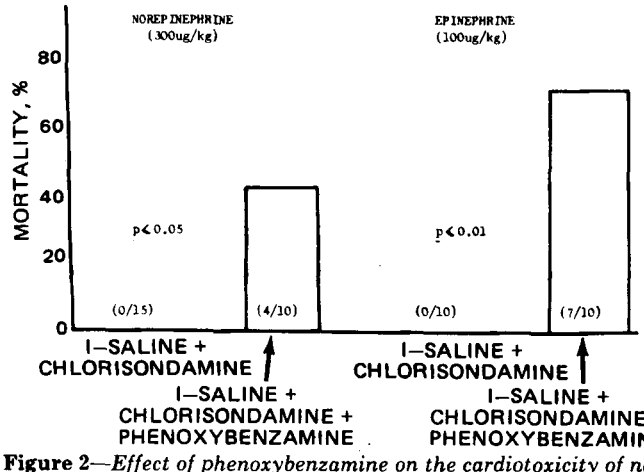

Figure 2—*Effect of phenoxybenzamine on the cardiotoxicity of norepinephrine and epinephrine in desoxycorticosterone acetate–saline-pretreated rats.*

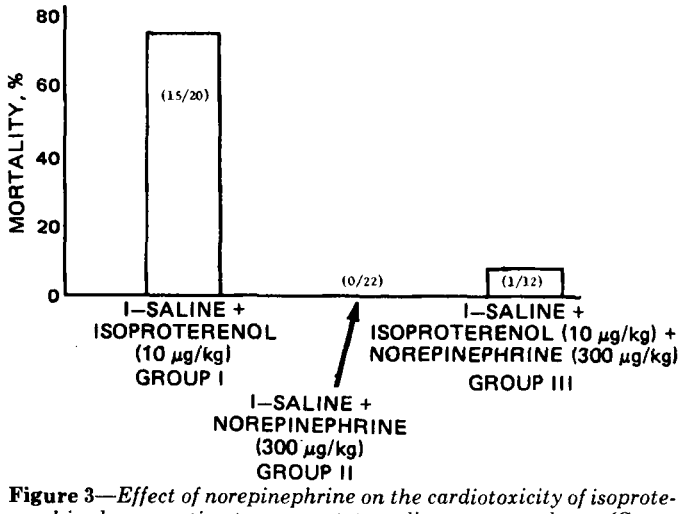

Figure 3—*Effect of norepinephrine on the cardiotoxicity of isoproterenol in desoxycorticosterone acetate–saline-pretreated rats (Group I* versus *Group III,* $p < 0.01$).

effect did, in fact, occur (Fig. 3). The addition of norepinephrine reduced the mortality rate attributable to isoproterenol alone from 75 to 8%.

DISCUSSION

Injections of norepinephrine and epinephrine can produce lethal cardiac arrhythmias in steroid-pretreated rats when combined with either aminophylline or the α-blocking agent phenoxybenzamine. The cardiotoxicity of isoproterenol, already established following steroid pretreatment alone, is enhanced greatly by aminophylline. Comparison of the LD_{50} value of isoproterenol in untreated rats to that of I–saline-pretreated rats also given aminophylline shows a decrease from 680 mg/kg to 3.06 μg/kg, a >200,000-fold increase in toxicity. The effect of aminophylline is so great that even the amounts of norepinephrine and epinephrine released endogenously as a stress response become lethal in its presence.

The ability of both phenoxybenzamine and aminophylline to increase the cardiotoxicity of adrenergic agonists may relate to the importance of β-adrenergic stimulation in this phenomenon. Norepinephrine and epinephrine contain potent α-adrenergic agonist activity in addition to their β-properties. Phenoxybenzamine and aminophylline each can upset, in a different way, the relative balance between α- and β-tone in favor of β-activity. The protective effect of α-stimulation may be due to its ability to raise the fibrillation threshold (9, 10). This effect would decrease the vulnerability of the heart to fibrillation induced by β-stimulation, reducing both the morbidity and mortality of the drug interaction.

Phenoxybenzamine may upset the α–β-balance by removing the α-component by receptor blockage. Aminophylline may produce its effect through its synergistic activity with β-adrenergic stimulation. This result might be accomplished through the adenylate cyclase system. β-Activity has been closely related to this system (11), and aminophylline, a potent phosphodiesterase inhibitor (12, 13), would prevent the degradation of formed adenosine-3′,5′-monophosphate (cyclic AMP) and enhance the activity of agents, such as β-agonists, whose actions depend on the elaboration of cyclic AMP. By either blocking α-receptors or enhancing β-stimulation, the net effect is to promote a β-preponderance, resulting in ventricular fibrillation.

The demonstrated beneficial effect of norepinephrine on isoproterenol cardiotoxicity is consistent with this theory. In this situation, potent α-adrenergic stimulation was added to an environment that was unbalanced favorably for β-adrenergic activity. By reestablishing a balance between α- and β-activity, the appearance of lethal arrhythmias was suppressed. The β-adrenergic component of norepinephrine may have played a small role in the diminution of the effect of isoproterenol. It is a weaker β-agonist than isoproterenol and, therefore, can act as a partial antagonist at certain dosage combinations with isoproterenol. However, this effect alone could not produce the observed decrease in mortality, from 75 to 8%. Hence, the potent α-activity of norepinephrine must have played the major role in decreasing the cardiotoxic response to isoproterenol.

The finding that endogenously released catecholamines can trigger ventricular fibrillation in steroid-pretreated rats fits well with current theories concerning the pathology of stress. Balazs *et al.* (14) found that

both cold and isolation stress increased the heart's sensitivity to isoproterenol and speculated that steroid hormones released by stress may have sensitized the myocardium to the β-agonist. Raab (15) also suggested that stress-induced discharge of adrenal corticoids may enhance the cardiotoxicity of endogenously released catecholamines. In discussing unexpected sudden death during sleep, he implicated a combination of 17-hydroxycorticosteroids, which reach peak levels during sleep, and dream-induced catecholamine discharges as being contributory to a fatal cardiotoxic interaction (16). The prolonged administration of steroids followed by exposure to catecholamines, as seen in the present study, may mimic the hormonal milieu produced by chronic stress, resulting in the similarity of toxic sequelae.

The applicability of these experimental findings to the clinical setting has to be determined. However, the data point out the potential danger of chronic steroid treatment. In such instances, there might be increased risk not only from administered catecholamines but also from agents that potentiate the effects of endogenous catecholamines, such as aminophylline and possibly monoamine oxidase inhibitors, and from agents that produce catecholamine discharge, such as tyramine, an ingredient of many foods.

REFERENCES

(1) G. Guideri, W. Goldman, and D. Lehr, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **30**, 686 (1971).

(2) G. Guideri, M. Barletta, and D. Lehr, *Cardiovasc. Res.*, **8**, 775 (1974).

(3) G. Rona, C. I. Chappel, T. Balazs, and R. Gaudry, *Arch. Pathol.*, **67**, 443 (1959).

(4) G. Guideri, M. Green, and D. Lehr, *Res. Commun. Chem. Pathol. Pharmacol.*, **21**, 197 (1978).

(5) D. Lehr, *N. Engl. J. Med.*, **287**, 987 (1972).

(6) G. Guideri, M. Barletta, R. Chau, M. Green, and D. Lehr, in "Recent Advances in Studies on Cardiac Structure and Metabolism, Vol. 10, The Metabolism of Contraction," P. E. Roy and G. Rona, Eds., University Park Press, Baltimore, Md., 1975, pp. 671–679.

(7) B. F. Hoffman and P. F. Cranefield, "Electrophysiology of the Heart," McGraw-Hill, New York, N.Y., 1960.

(8) G. Guideri, C. Healy, and D. Lehr, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **37**, 817 (1978).

(9) J. G. Papp and L. Szekeres, *Eur. J. Pharmacol.*, **3**, 4 (1968).

(10) A. J. Pappano, *J. Pharmacol. Exp. Ther.*, **177**, 85 (1971).

(11) E. W. Sutherland and T. W. Rall, *Pharmacol. Rev.*, **12**, 265 (1960).

(12) E. W. Sutherland and T. W. Rall, *J. Biol. Chem.*, **232**, 1077 (1958).

(13) R. W. Butcher, Jr., and E. W. Sutherland, *Pharmacologist*, **1**, 63 (1959).

(14) T. Balazs, J. B. Murphy, and H. C. Grice, *J. Pharm. Pharmacol.*, **14**, 750 (1962).

(15) W. Raab, *Am. Heart J.*, **72**, 538 (1966).

(16) W. Raab, in "Recent Advances in Studies on Cardiac Structure and Metabolism, Vol. 1, Myocardiology," E. Bajusz and G. Rona, Eds., University Park Press, Baltimore, Md., 1970, p. 707.

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Application of High-Pressure Liquid Chromatography and Thermal Energy Analyzer to Analysis of Trinitroglycerin and Its Metabolites in Blood

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Abstract □ A highly selective and sensitive analytical procedure for the determination of trinitroglycerin and four metabolites in whole blood was developed. Trinitroglycerin and its metabolites were extracted from whole blood with ethyl acetate and analyzed by high-pressure liquid chromatography using the thermal energy analyzer detector. Linearity of response was observed over the 1–1000-ng range. The applicability of this method to the analysis of whole blood from dogs orally dosed with trinitroglycerin is described.

Keyphrases □ Trinitroglycerin—high-pressure liquid chromatographic analysis of parent drug and metabolites in whole blood □ High-pressure liquid chromatography—analysis of trinitroglycerin and metabolites in whole blood □ Thermal energy analyzer—analysis of trinitroglycerin and metabolites in whole blood

The analysis of trinitroglycerin in blood has been performed primarily by electron-capture GLC (1–3). Although this method possesses high sensitivity and some selectivity for trinitroglycerin, it requires extensive cleanup procedures and solvent purification. Furthermore, it does not adequately account for the primary metabolites of trinitroglycerin, the isomeric mono- and dinitroglyceryl esters. A recent report (4) described the detection of ethylene glycol dinitrate esters in water by use of the thermal energy analyzer. It was thought that the analyzer detection system also may be applicable to the analysis of trinitroglycerin in complex matrixes such as whole blood.

This report describes the development of a high-pressure liquid chromatographic (HPLC) method to determine trinitroglycerin as well as the primary isomeric dinitro- and mononitroglyceryl metabolites in whole blood. The application of this methodology to the blood of dogs orally dosed with trinitroglycerin is described.

EXPERIMENTAL

Reagents—Acetone[1] and ethyl acetate[1] were glass distilled. Trinitroglycerin[2] was used as a powder (10% trinitroglycerin in lactose). *N*-Nitrosodipropylamine[3] was supplied as a standard solution in isooctane. All other chemicals and solvents were standard reagent grade and were used without further purification. Authentic samples of 1,3- and 1,2-dinitroglycerols and 1- and 2-mononitroglycerols were obtained[4].

Apparatus—The chromatographic system consisted of a liquid

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