cause backing up of urine. This explanation is untenable since the presence of such plugs depends on the method of killing, and because such plugs form at time of death. Silverstein *et al.* did not find hydronephrosis in control or polydypsic female mice. Therefore, the obstructive phenomenon in their animals may well depend on anatomical differences between male and female in the lower urinary tract, but this difference is not the urethral plug, for the reasons given above.

The above facts also must be considered in experiments in which the weight of the seminal vesicles is used as an index of androgen activity. It should be kept in mind that killing animals with sodium pentobarbital prevents the loss of seminal fluid that accompanies the formation of urethral plugs.

The significance of urethral plugs was properly interpreted by Engle(2) who wrote: "Those who have worked with laboratory rodents know that a tough coagulum usually is found in the urethra of a male which is killed by a sudden blow on the head, and also after death by illuminating gas, especially if the animal died struggling."

Summary. The terminal formation of urethral plugs of coagulated seminal fluid in male mice depended on the method of killing. Plugs were found when mice were killed by ether inhalation, but not when killed with sodium pentobarbital injected subcutaneously. These plugs were produced by an abnormal ejaculation during a terminal convulsion which occurred in mice killed by ether, but not in sodium pentobarbital-killed mice. High lumbar cord transection prevented plug formation. Normal ejaculation did not produce urethral plugs. It was concluded that such urethral plugs are not a natural cause for urinary obstruction since they form terminally.

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Nitrites XX. Pharmacologic Studies with 1-Ethylglyceryl Trinitrate. (27756)

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For a number of years we have been investigating the structure-activity relationships of compounds related to glyceryl trinitrate. It was shown that the number of nitrate groups in the molecule was not a salient factor in determining vasodilating potency. Furthermore the activity was dependent upon the intact molecule of the ester(1). These studies are concerned with the activity of 1-ethylglyceryl trinitrate (EGT)(1,2,3-Pentanetriol-trinitrate). This ester is identical with glyceryl trinitrate with the exception that one of the hydrogen atoms on C^1 has been replaced by an ethyl group.

Methods. Coronary blood flow was determined by the modified Langendorff(2) procedure using rabbits' hearts.

Studies were conducted on the fate of EGT and glyceryl trinitrate upon passage through the heart in a Langendorff preparation omitting the glucose from the perfusion fluid. Total ester and nitrite were determined in the perfusion fluid to which each ester had been added respectively. The same procedure was carried out by adding the respective esters to the perfusion fluid and determining the total ester and nitrite content at definite time periods in the perfusates.

The hydrolysis studies of the nitrate esters and their effect upon blood pressure were

TABLE I. Comparison of Effects of EGT and Glyceryl Trinitrate on Coronary Flow in Isolated Rabbit Heart.*

Compound	Dose	0.1 mg		0.5 mg		1 mg	
		Effect					
		% change	Recovery, sec.	% change	Recovery, sec.	% change	Recovery, sec.
EGT	High	+50.0	100.0	+71.0	120.0	+107.0	120.0
	Low	+ 4.0	40.0	+12.0	55.0	+ 10.0	45.0
	Mean	+30.0	69.0	+45.0	86.0	+ 53.0	76.0
	S.D.	±15.4	±18.4	±16.9	±22.0	± 23.9	±21.9
Glyceryl trinitrate	High	+60.0	95.0	+72.0	120.0	+104.0	100.0
	Low	+ 4.0	45.0	+ 3.0	45.0	+ 11.0	45.0
	Mean	+32.0	71.0	+43.0	86.0	+ 52.0	82.0
	S.D.†	±20.1	±16.7	±18.3	±24.4	± 24.7	±18.9

* Results obtained from 10 hearts.

† The differences between the paired percentage changes in coronary flow and duration of action of the 2 esters are not statistically significant ($p > 0.5$).

conducted according to the procedure of Krantz *et al.*(1).

The oil/water coefficient was determined by the method employed by Burgison *et al.* (3).

Comparative rates of hydrolysis were determined *in vitro*. Each ester was dissolved in acetone at a concentration of 0.1 M. One volume of the ester solution and 2 volumes of 0.2 N NaOH were mixed and incubated at 37°C. Aliquots were removed at various periods and titrated with 0.1 N HCl.

The effect of EGT on the blood pressure of the dog was determined by intravenous injection in the animal under pentobarbital sodium anesthesia. The dose was 0.75 ml/kg of a $1:10^4$ solution. The procedure used for alkaline hydrolysis was carried out according to Krantz *et al.*(1). The products of hydrolysis were injected, after neutralization, in the same manner.

EGT was synthesized in this laboratory by the nitration of ethyl glycerin (1,2,3-pentanetriol)(4), using equal volumes of concentrated sulfuric acid and red fuming nitric acid (d.1.60) at −5 to 0°C. It is a pale yellow oil, Sp. G. 1.4377^{20}_{20}, n^{20}_{D} 1.4570. It is miscible with alcohol and acetone in all proportions and very slightly soluble in water.

The glyceryl trinitrate was of U.S.P. quality.

Results. Table I shows the comparative activity of EGT and glyceryl trinitrate on the coronary vessels of the rabbit's heart.

Table II shows the relative quantities of EGT and glyceryl trinitrate fixed by the cardiac tissue and the degree of nitrite formation during perfusion.

Table III shows the percentage fall in blood pressure upon intravenous injection of EGT before and after boiling with 0.1 N NaOH.

Table IV shows the effect of alkaline hy-

TABLE II. EGT and Glyceryl Trinitrate Recovered from Perfused Heart and Reduced to Nitrite.*

	EGT			Glyceryl trinitrate		
	Vol, ml†	Total ester, μg	Total NO_2^-, μg	Vol, ml†	Total ester, μg	Total NO_2^-, μg
High	82	470	.0	77	477	7.5
Low	49	422	"	31	381	2.8
Mean	63	451.3	"	54.9	429	5.0
S.D.	±11.9	±19.5	"	±14.8	±33.9	±1.5

Results obtained from 8 hearts.

* Dose of each ester 500 μg.

† Data were obtained by collecting the perfusate for 2 min. immediately after administration of the ester. Approximately 88% of initial dose was recovered in this period. The method is sensitive to 0.2 μg of NO_2^-.

TABLE III. Effect of Alkaline Hydrolysis on Vasodepressor Action of Ethylglyceryl Trinitrate.

Dog			EGT			EGT after hydrolysis		
			Mean arterial pressure, mm Hg			Mean arterial pressure, mm Hg		
Wt, kg	Sex	Trial	Before	After	% change	Before	After	% change
7.4	♀	1	232	196	−15.5	224	226	+1.0
		2	234	210	−10.3	226	228	+1.0
17.6	♂	1	178	104	−41.5	170	170	0
		2	172	144	−16.5	170	170	0
7.9	♀	1	136	88	−35.3	126	130	+3.2
		2	124	106	−14.5	128	130	+1.8

drolysis on the two nitrate esters.

The oil/water coefficient of EGT was found to be 97 ± 3; that of glyceryl trinitrate determined under the same conditions is 109 ± 3.

Discussion. The data in Table I show that EGT is equipotent as a coronary dilator of the rabbit's heart as glyceryl trinitrate. Their oil/water coefficients differ by only 12 units. The quantities of each compound recovered in the coronary perfusion experiments are approximately the same, indicating the same degree of fixation by cardiac tissue for each ester. During the passage of EGT through the coronary vessels, no measurable amount of nitrate was reduced to nitrite. However with glyceryl trinitrate significant reduction to nitrite occurs. Like glyceryl trinitrate, EGT evokes a depressor response when injected intravenously into dogs. The degree and duration of vasodepressor response to EGT is slightly less than that evoked by glyceryl trinitrate. The response of EGT is nullified by boiling with alkali as was previously shown to occur with glyceryl trinitrate(1).

TABLE IV. Alkaline Hydrolysis of EGT and Glyceryl Trinitrate.

	% hydrolysis	
Time, min.	EGT	Glyceryl trinitrate
1	32	68
2		89
3	35	98
4		100
5	37	"
10	38	
20	40	
60	45	

The data in Table IV show that the behavior of EGT and glyceryl trinitrate to alkaline hydrolysis is quite different. Approximately 30% of EGT is hydrolyzed within 1 minute, however the remaining nitrate ester is relatively refractory to hydrolysis with alkali at 37°C. These data suggest that EGT undergoes rapid hydrolytic cleavage of one nitrate group and further degradation is a slow process. Previously(1) it was shown that the activity of nitrated glyceryl esters is dependent upon the intact molecule. The vasodepressor response of isosorbide dinitrate, which is refractory to hydrolysis at 100°C, is not modified by this treatment(5).

Summary. It has been shown that 1-ethylglyceryl trinitrate qualitatively and quantitatively evokes the same character of coronary dilatation as does glyceryl trinitrate. Evidence has been produced to show that EGT elicits its dilating response by the activity of the intact ester. Upon alkaline hydrolysis with concomitant formation of nitrite ion its vasodepressor action is nullified.

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