The Effects of Nitroglycerin and Amyl Nitrite on Arteriolar and Venous Tone in the Human Forearm

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IN SPITE OF the widespread use of nitroglycerin and amyl nitrite for relieving the pain of angina pectoris, considerable controversy still surrounds the mechanism of action of these two drugs. It has been proposed that they improve the relationship between coronary blood flow and the oxygen requirements of the heart by a direct dilating action on the coronary arteries,1-4 or that they diminish the oxygen requirements of the myocardium by diminishing the external work of the heart.5, 6 Much of the confusion concerning the actions of these drugs has arisen from the assumption that nitroglycerin and amyl nitrite have similar effects. Moreover, many studies on the peripheral circulatory effects of nitrates have been carried out with sodium nitrite, administered orally or intravenously, and it has generally been considered that the actions of this substance resemble those of nitroglycerin and of amyl nitrite. In order to determine whether the peripheral circulatory actions of nitroglycerin and amyl nitrite might play a role in their therapeutic effects, the actions of these drugs on both the resistance and capacitance vessels of the forearm of normal human subjects were determined, and the results of these studies are described in this report. The effects of nitroglycerin on ventricular dimensions are described elsewhere.7 The results of these two investigations suggest the manner in which the actions of these drugs on the peripheral circulation affect the heart, and they afford additional insight into the mechanism by which nitroglycerin and amyl nitrite provide relief of anginal pain.

Methods

A total of 11 normal male subjects, ranging in age from 18 to 50 years, were investigated. Studies with both amyl nitrite and nitroglycerin were carried out on the forearms of nine of these subjects. All experiments were carried out with the subjects in the supine position, with the forearm elevated so that venous pressure was zero, and the possible effects of the drugs on the vessels of the hand were eliminated by inflating a wrist cuff to suprasystolic pressure levels before each measurement. A venous occlusion plethysmographic technic, employing the Whitney mercury-in-rubber strain-gauge plethysmograph,8 placed on the midforearm, was used for the measurement of forearm blood flow, as described previously.9, 10 A sphygmomanometric cuff was placed around the upper arm and venous outflow from the forearm was occluded by suddenly inflating this cuff to a pressure below the diastolic arterial pressure. Forearm blood flow was calculated from the change in forearm circumference during venous occlusion, and was expressed in ml. per 100 Gm. tissue per minute.8-10 Arterial pressure was measured through an indwelling needle placed into the brachial artery of the opposite forearm, and forearm vascular resistance was calculated as the ratio of mean arterial pressure to forearm blood flow and expressed in units of \( \frac{\text{mm. Hg}}{\text{ml./100 Gm./min.}} \).

The effects of the drugs on the venous bed of the forearm were determined by an acute occlusion method and by several equilibration technics. The acute occlusion method has been described previously11-13; forearm venous pressure was measured through a catheter introduced into a vein at the wrist and advanced so that its tip lay just distal to the mercury-in-rubber gauge. The pressure-volume characteristics of the capacitance vessels were calculated by determining the ratio of the increment in venous pressure to the increment in forearm volume which occurred in the 10 seconds following inflation of the venous occlusion cuff, and were expressed in units of mm. Hg per ml.; this ratio was not altered significantly by the precise time during the first 30 seconds...
after occlusion at which the measurements were made.

The first and second equilibration methods have also been described previously.\(^{12, 13}\) In the first, the venous cuff was suddenly inflated to 30 mm. Hg, and venous pressure and forearm circumference were permitted to equilibrate for 2 minutes. The tone of the capacitance vessels was calculated from the increments in volume and pressure that had taken place during the full 2-minute period. The second method was a modification of the stepwise venous occlusion technique described by Wood and Eckstein\(^ {14}\); the venous occluding cuff was inflated in 5-mm. Hg increments to 30 mm. Hg and sufficient time was permitted to elapse between each of these inflations for the venous pressure and forearm circumference to reach stable values, thus allowing the construction of venous pressure-volume curves.

In the third equilibration method the venous occlusion cuff was inflated to 30 mm. Hg, and venous pressure and forearm circumference were permitted to equilibrate for 2 minutes. Under these conditions the veins were distended, the forearm venous pressure remaining stable at 30 mm. Hg. The drug was then administered and the induced changes in the volume of blood in the capacitance vessels were continuously assessed by measuring forearm circumference plethysmographically; a decrease in limb circumference indicated venoconstriction, and an increase signified venodilatation. Utilizing this technic venous tone was also expressed in units of mm. Hg per ml. per 100 Gm., and calculated by determining the ratio of the increment in forearm venous pressure (30 mm. Hg), to the increment in forearm volume that had occurred at any time after equilibration had taken place with the venous occluding cuff inflated. This calculation neglects the small increment in forearm volume consequent to transudation of fluid into the interstitial space. This method differs from the first equilibration method in that the drug is administered after the venous occlusion.

In the fourth equilibration method the volume

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**Figure 1**

*Average change (± S.E.M.) of mean arterial pressure (MAP), forearm blood flow (F.B.F.), forearm vascular resistance (F.V.R.), and forearm venous tone, determined by the acute occlusion method, following nitroglycerin (NTG), expressed as percentage changes from the control values in eight subjects. Average S.E.M. = standard error of the mean of the four measurements during the control period carried out in any one subject, averaged for the eight subjects, and expressed as a percentage of the mean.*

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of the capacitance vessels was assessed as just described, except that venous occlusion was not carried out. This technic simply allows determination of the effects of the drugs on the volume of blood contained within the capacitance vessels of the forearm at the prevailing venous pressure.

All studies were performed with the subjects in the basal, postabsorptive state. Following placement of the catheter and needles, four control acute venous occlusion curves were obtained at 2-minute intervals. Amyl nitrite was then inhaled for 30 seconds from a broken ampule held over the nose, and venous occlusion curves were recorded at 15-second intervals during the next 3 minutes. Fifteen minutes later amyl nitrite was readministered and the effects of the drug on venous tone were assessed by one of the equilibration technics. After another 15 minutes, 0.6 to 0.9 mg. of nitroglycerin were administered sublingually, and the effects on arterial and venous tone were assessed by both the acute occlusion and equilibration technics.

In six of the subjects the actions of amyl nitrite, and in four of the subjects the effects of nitroglycerin were also studied 20 minutes after the intravenous administration of 0.5 mg. per Kg. of guanethidine. In addition, the effects of each drug were studied in two subjects both before and after the oral administration of reserpine, 0.50 mg. daily for 3 and 12 months, respectively. The drugs had produced adrenergic blockade, as evidenced by abolition of the post-Valsalva systolic arterial pressure overshoot, and by the marked reduction or absence of the increase in forearm vascular resistance and venous tone which normally occurs during the cold pressor test.13

Results

Nitroglycerin

Mean arterial pressure declined slightly and forearm blood flow increased in all eight subjects in whom serial measurements were

Two segments of a recording obtained by the acute occlusion method. The tracing on the left was recorded during the control period and that on the right 2 minutes after the sublingual administration of 0.9 mg. of nitroglycerin. PLETH. refers to the forearm plethysmographic tracing. VP = forearm venous pressure. The figures below the tracing are the values of the variables that were measured or calculated. HR = heart rate. FVT = forearm venous tone. For other abbreviations see legend of figure 1. Note that after nitroglycerin the rise in the plethysmographic tracing of forearm circumference was more rapid than during the control period, while the rise in forearm venous pressure was almost unchanged, indicating that venodilatation had occurred.

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A. Venous tone measurements carried out 2 minutes after inflation of the occlusion cuff (Equilibration Method 1). The solid bars represent 3 values during the control period. The cross-hatched bars represent measurements made at various time intervals after 0.9 mg nitroglycerin. B. Relationship between venous pressure and forearm volume increase, determined by the stepwise occlusion method (Equilibration Method 2), during the control period and after nitroglycerin.

made after the administration of nitroglycerin; the calculated forearm vascular resistance therefore decreased, by an average of 35 per cent of the control values. Similarly, venous tone determined by the acute occlusion method declined in each subject, the fall averaging 19 per cent of control (figs. 1 and 2). The changes in all four of these variables were statistically significant \( p < 0.01 \). In the four subjects in whom the effects of nitroglycerin were redetermined after intravenous guanethidine, it was observed that adrenergic blockade had not affected the directional results in any of the measured variables. However, the reduction of forearm vascular resistance produced by nitroglycerin was significantly greater \( p < 0.05 \) after guanethidine. Reserpine administration in two subjects did not affect the actions of nitroglycerin on the circulatory dynamics of the forearm.

In all eight subjects in whom the effects of nitroglycerin on venous tone were measured by the first and second equilibration methods, a decrease in venous tone occurred (fig. 3). The venous tone, determined 2 minutes after venous occlusion (method 1, fig. 3A) fell within 5 minutes in all subjects by an average value of \( 1.3 \pm 0.3 \text{ mm. Hg per ml} \). During the subsequent 20 minutes the venous tone gradually returned to control values. Similarly, when venous tone was determined by the stepwise occlusion method (method 2, fig. 3B), nitroglycerin resulted in a fall of \( 1.1 \pm 0.4 \text{ mm. Hg per ml} \).

In the subject in whom the effects of nitroglycerin were assessed continuously by the third equilibration method (venous pressure held constant at 30 mm. Hg) nitroglycerin resulted in an increase in forearm circumference, indicating that venodilatation had occurred (fig. 4A). In the subject in whom changes in the forearm circumference were measured continuously without venous occlusion, nitroglycerin also resulted in an increase in forearm volume, indicating that a displacement of blood into the forearm had occurred (fig. 4B). As was observed with the acute occlusion method, neither the administration of guanethidine (four subjects) nor reserpine (two subjects) affected the action of nitroglycerin on the venous bed of the forearm as determined by any of the equilibration methods.

**Amyl Nitrite**

In all 10 subjects given amyl nitrite, mean arterial pressure declined immediately, reaching a nadir, which averaged 45 per cent of the control pressure 30 seconds after the beginning of the inhalation. Forearm blood flow rose steadily to a peak, which averaged 86 per cent above control 75 seconds after the onset of inhalation. As a consequence, calculated
forearm vascular resistance declined, falling to an average value 58 per cent below control 45 seconds after inhalation. Venous tone, determined by the acute occlusion method, rose markedly to peak values which averaged 221 per cent above control 45 seconds after inhalation (figs. 5 and 6). In six of these 10 subjects, the effects of amyl nitrite were repeated after the intravenous administration of guanethidine (figs. 7 and 8). The extent of the reduction of forearm vascular resistance induced by amyl nitrite was significantly greater after guanethidine ($p < 0.05$, fig. 8A), while the increase in venous tone, measured by the acute occlusion method, was significantly diminished ($p < 0.01$, fig. 8B). In two subjects reserpine also attenuated or abolished the vеноconstrictor response to amyl nitrite, venous tone increasing 16.9 and 6.7 mm. Hg per ml., respectively, before reserpine and 1.2 and 0 mm. Hg per ml. afterwards.

In the subject in whom changes in the fore-
Arm circumference were measured continuously without venous occlusion (Equilibration Method No. 4), the inhalation of amyl nitrite resulted in a diminution of forearm volume, indicating that blood had been displaced out of the forearm (fig. 9). In nine subjects the effects of amyl nitrite on venous tone were determined by the third equilibration method, i.e., measuring forearm circumference continuously before and after administering the drug, while forearm venous pressure was held constant at 30 mm. Hg (fig. 10). Shortly after the precipitous fall in arterial pressure had begun, a marked decrease in forearm volume was noted, indicating that intense venoconstriction had taken place, the peak increase in venous tone, measured by this technic, averaging 43 per cent above control values. Arterial pressure began to return to normal shortly before the peak venoconstrictor response had occurred. In six of these subjects the effects of amyl nitrite were remeasured by this technic after guanethidine. As noted with the acute occlusion method, the venoconstriction induced by amyl nitrite was markedly and significantly attenuated (p < 0.01, fig. 10B and C) even though the decline in arterial pressure was more pronounced (fig. 10B). Similarly, reserpine reduced the augmentation of venous tone resulting from amyl nitrite. These increases, measured by the third equilibration method, equaled 6.0 and 11.8

**Figure 5**

Average change (± S.E.M.) of mean arterial pressure, forearm blood flow, forearm vascular resistance, and venous tone in 10 subjects following the inhalation of amyl nitrite (A.N.), expressed as percentage changes from the control values. For explanation and abbreviations see legend to figure 1.
mm. Hg per ml., respectively, prior to reserpine, and 3.2 and 3.5 mm. Hg per ml. afterwards.

**Discussion**

Although it is agreed that the principal pharmacologic action of nitrates is to dilate vascular smooth muscle, the mechanism by which these drugs relieve the pain of coronary ischemia is still debated. Studies carried out in vitro have shown that the nitrates relax coronary arterial strips, and that nitroglycerin diminishes coronary vascular resistance in the isolated canine heart. There is also evidence that nitroglycerin increases myocardial blood flow in intact man. However, on the basis of experiments utilizing a variety of different technics for measuring coronary blood flow it has been concluded that this drug does not regularly increase this flow in patients with coronary artery disease, although it does reduce arterial pressure. The fundamental question of whether or not nitrates and organic nitrates increase the blood flow to the ischemic portion of the myocardium in patients with coronary atherosclerosis has not been resolved.

The possibility has also been considered that the therapeutic action of these drugs may in part be dependent upon their action on the peripheral circulation. Considerable evidence has been obtained from experimental animals that nitrates administered intravenously dilate cutaneous, retinal, meningeal, and splanchnic vessels. However, there is relatively little information concerning the action of these drugs on the peripheral vessels in intact human subjects. In 1937 Wilkins, Haynes, and Weiss showed that 120 to 180 mg. of sodium nitrite, administered orally, tended to dilate the veins of the hand, although at normal pressures the volume of the hand remained essentially unchanged. Artiolar tone was usually not altered, and in some studies these investigators even found that it increased. More recently, Ablad and Johnsson infused sodium nitrite continuously into the brachial artery and noted that although forearm vascular resistance diminished, the major effect of the drug was to dilate the post-capillary bed.

It is generally appreciated that drugs having a direct effect upon blood vessels may also evoke powerful reflex responses when they
are administered systemically and, in an intact organism, the observed action of the drug is often a combination of direct and reflex effects. Since the various nitrites and nitrates differ in their speed and intensity of action, depending on the specific drug and its route of administration, it was felt that rather than studying sodium nitrite, which is not used in the treatment of angina pectoris, the effects of nitroglycerin and amyl nitrite themselves on the peripheral circulation should be investigated. Further, it was thought that these drugs should be administered by the routes that are employed in clinical practice, i.e., sublingually and by inhalation, respectively.

Nitroglycerin resulted in a mild decline in systemic arterial pressure and a concomitant elevation of forearm blood flow, and therefore a decrease in the calculated forearm vascular resistance. These changes developed over the course of 2 to 5 minutes after administration and then gradually regressed during the following 20 to 30 minutes. In the interpretation of these results it is pertinent that measurements of forearm blood flow, vascular resistance and venous tone during a 40-minute period in which no drug was administered resulted in no consistent or significant changes in these variables; thus the changes that were observed may be attributed to the administration of nitroglycerin. Although cardiac output was not measured in this investigation, other studies in this laboratory\textsuperscript{7, 27} have indicated that sublingual nitroglycerin generally diminishes total blood flow slightly. If the increases in blood flow which were observed in the forearms occurred in other skeletal muscle groups, then it would appear that substantial redistribution of regional blood flow must occur following nitroglycerin. The finding that adrenergic blockade with guanethidine accentuated the decline in resistance produced by nitroglycerin suggests that when the drug is given in the presence of a normally functioning sympathetic nervous system, the fall in resistance is partially opposed by sympathetically induced reflex arteriolar constriction.

It was observed with the acute occlusion technic and with several equilibration methods by which venous tone was measured, that

\begin{figure}
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\includegraphics[width=\textwidth]{figure7.png}
\caption{Figure 7}
\end{figure}

Two segments of recordings following adrenergic blockade with guanethidine obtained in the same subject whose tracings are shown in figure 6. Amyl nitrite increased the rate of rise of forearm circumference proportionately more than the rate of rise of venous pressure after guanethidine than before, indicating that the vеноconstriction which had occurred was less than that noted before guanethidine had been given (figure 6).

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simultaneously with the fall in vascular resistance, nitroglycerin dilated the capacitance vessels of the forearm. Significant pooling of blood in the forearm was observed both when venous pressure was controlled (fig. 4A) and when it was permitted to change (fig. 4B). These findings of venodilatation and pooling of blood provide an explanation for the fall in cardiac output which occurs after nitroglycerin, for the syncopal episodes which are occasionally seen, as well as for the decreases which this drug induces in the pulmonary artery and capillary pressures and the a wave of the apex cardiogram. Similarly the venous and arteriolar dilatation induced by nitroglycerin may also account for the diminution of ventricular size which occurs, as described elsewhere. This decrease in ventricular size represents a mechanism by which the drug may reduce myocardial oxygen requirements and thereby relieve the pain of angina pectoris. The venodilatation produced by nitroglycerin and the resultant reduction of ventricular systolic dimensions also explains the intensification of obstruction to left ventricular outflow which this drug induces in patients with idiopathic hypertrophic subaortic stenosis.

The effects of inhaling amyl nitrite differed strikingly from those observed after nitroglycerin. Arterial pressure fell precipitously, while blood flow increased, indicating that a marked fall in forearm vascular resistance had
A and B. Recordings of arterial and venous pressures and forearm circumference following amyl nitrite inhalation (Equilibration, Method 3). Tracing A was recorded during the control period and B after guanethidine. After adrenergic blockade (B) amyl nitrite decreased forearm volume considerably less than during the control period (A), indicating that venoconstriction was markedly diminished. C. Average values in six subjects showing the effects of amyl nitrite on forearm venous tone, estimated by equilibration method 3, during the control period (solid dots, solid lines) and after guanethidine (open circles, broken lines).

occurred. This decline, although relatively brief, was much more intense than that observed following sublingual nitroglycerin. The decreases in arterial pressure and calculated vascular resistance which occurred were opposed by the activity of the sympathetic nervous system, since after adrenergic blockade had been induced, amyl nitrite resulted in even greater reductions in these two variables. In contrast to nitroglycerin, amyl nitrite induced a pronounced venoconstriction in every subject, as determined both with the acute occlusion and equilibration technics. The finding that the venoconstriction commenced with the arterial pressure decline and was most marked immediately after the nadir of the pressure fall (fig. 10A), suggests that this venoconstrictor response was reflex in origin and in part due to a decrease in pressure acting upon the baroreceptors. In order to clarify the possible role of venoconstriction in the response to amyl nitrite, this agent was administered to subjects in whom adrenergic blockade had been induced with guanethidine or reserpine. The reflex venoconstriction which normally occurs during the cold pressor test had been eliminated or reduced by these blocking agents, and they also abolished or markedly attenuated the venoconstrictor response to amyl nitrite, thus providing further support for

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the hypothesis that this response is reflex in origin and that it is mediated through the efferent sympathetic nerves. It is now well established that amyl nitrite induces a substantial elevation of cardiac output\textsuperscript{[31,32]} and it is likely that the profound venoconstriction demonstrated in this investigation plays an important role in this response.

Since amyl nitrite and nitroglycerin have directionally opposite effects on the venous bed, the efficacy of both of these drugs in angina pectoris can obviously not be explained by their effects on veins alone. Inhaled amyl nitrite results in a sudden and marked arteriolar dilatation, and it is probable that the coronary vessels participate in this response. As a consequence of this arteriolar dilatation, systemic pressure declines markedly, thus also reducing myocardial oxygen requirements.\textsuperscript{33} It seems likely that the salutary clinical effects of this drug result from a combination of these two actions.

**Summary**

The effects of sublingual nitroglycerin and inhaled amyl nitrite on the arteriolar and venous beds of the forearm were studied in 11 normal subjects. Forearm blood flow was measured with a strain-gauge plethysmograph, and venous tone was determined both by an acute occlusion technic, and by several equilibration technics. Nitroglycerin reduced systemic arterial pressure, elevated forearm blood flow, lowered forearm vascular resistance and decreased venous tone. Amyl nitrite diminished arterial pressure, elevated forearm blood flow, markedly decreased forearm vascular resistance, but in contrast to nitroglycerin augmented venous tone strikingly. This venoconstriction was abolished or diminished when adrenergic activity was impeded by administration of either guanethidine or reserpine.

**References**


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The blood, therefore, after thinning, passes via the vena-arterialis (pulmonary artery) to the lung for circulation and mixes with air in the pulmonary parenchyma. The aerated blood gets refined and passes through the arteria-venalis (pulmonary vein) to reach the left cavity of the two cavities of the heart, after having mixed with the air and become suitable for the evolution of the animal spirit.—André Cournand, M.D. Circulation of the Blood. Edited by Alfred P. Fishman, M.D., and Dickinson W. Richards, M.D. New York, Oxford University Press, 1964, p. 16.
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