stroke volume at rest and during exercise in human beings. J Clin Invest 30:1208, 1960

43. Chapman CB, Baker O, Mitchell JH: Left ventricular function at rest and during exercise. J Clin Invest 38:1202, 1959

44. Mitchell JH, Sproule BJ, Chapman CB: The physiological meaning of the maximal oxygen intake test. J Clin Invest 37:538, 1958

45. Bevegard S, Holmgren A, Jonsson B: Effect of body position on the circulation at rest and during exercise, with special reference to the influence on the stroke volume. Acta Physiol Scand 49:279, 1960

46. Epstein SE, Robinson BF, Kahler RL, et al: Effects of beta-adrenergic blockade on the cardiac response to maximal and submaximal exercise in man. J Clin Invest 44:1745, 1965

47. Robinson BF, Epstein SE, Kahler RL, et al: Circulatory effects of acute expansion of blood volume: studies during maximal exercise and at rest. Circ Res 19:26, 1966

48. Bainbridge FA: The Physiology of Muscular Exercise. London, Longmans, Green, 1919

49. Means JH, Newburgh LH: The effect of caffeine upon the blood flow in normal human subjects. J Pharmacol Exp Ther 7:449, 1915

50. Lindhard J: Ueber die Regulierung des Kreislaufes im gesunden und kranken Organismus. Cardiologia 1:366, 1937

51. Franklin DL, Ellis RM, Rushmer RF: Aortic blood flow in dogs during treadmill exercise. J Appl Physiol 14:809, 1959

52. Rushmer RF, Smith O, Franklin D: Mechanisms of cardiac control in exercise. Circ Res 7:602, 1959

53. Sonnenblick EH, Braunwald E, Williams JF Jr, et al: Effects of exercise on myocardial force–velocity relations in intact unanesthetized man: relative roles of changes in heart rate, sympathetic activity, and ventricular dimensions. J Clin Invest 44:2051, 1965

54. Braunwald E, Goldblatt A, Harrison DC, et al: Studies on cardiac dimensions in intact, unanesthetized man. III. Effects of muscular exercise. Circ Res 13:460, 1963

55. Gorlin R, Cohen LS, Elliott WC, et al: Effect of supine exercise on left ventricular volume and oxygen consumption in man. Circulation 32:361, 1965

56. Kjellberg SR, Rudhe U, Sjostrand T: The amount of hemoglobin (blood volume) in relation to the pulse rate and heart volume during work. Acta Physiol Scand 19:152, 1950

57. Bruce TA, Chapman CB, Baker O, et al: The role of autonomic and myocardial factors in cardiac control. J Clin Invest 42:721, 1963

58. Braunwald E, Sonnenblick EH, Ross J Jr, et al: An analysis of the cardiac response to exercise. Circ Res 20(Suppl I):44, 1967

59. Patrick TA, Vatner SF, Kemper WS, et al: Telemetry of left ventricular diameter and pressure measurements from unrestrained animals. J Appl Physiol 37:276, 1974

60. Horwitz LD, Atkins JM, Leshin SJ: Effect of beta adrenergic blockade on left ventricular function in exercise. Am J Physiol 227:839, 1974

61. Vatner SF: Response of the failing heart to severe exercise. Clin Res 24:422A, 1976

62. Rapaport E, Wong M, Ferguson RE, et al: Right ventricular volumes in patients with and without heart failure. Circulation 31:531, 1965

63. Vatner SF, McRitchie RJ, Maroko PR, et al: Effects of cathecholamines, exercise, and nitroglycerin on the normal and ischemic myocardium in conscious dogs. J Clin Invest 54:563, 1974

64. Berne RM: Regulation of coronary blood flow. Physiol Rev 44:1, 1964

65. Braunwald E: Control of myocardial oxygen consumption: Physiologic and clinical consideration. Am J Cardiol 27:416, 1971

66. Vatner SF, Higgins CB, Franklin D, et al: Role of tachycardia in mediating the coronary hemodynamic response to severe exercise. J Appl Physiol 32:380, 1972

67. Van Citters RL, Franklin DL: Cardiovascular performance of Alaska sled dogs during exercise. Circ Res 24:33, 1969

68. Khouri EM, Gregg DE, Rayford CR: Effects of exercise on cardiac output, left coronary flow and myocardial metabolism in the unanesthetized dog. Circ Res 17:427, 1965

69. Robinson S: Physiology of muscular exercise, in Mountcastle VB (ed): Medical Physiology. Saint Louis, Mosby, 1974, p 1273

70. Johnson PC: The microcirculation, and local and humoral control of the circulation, in Guyton AC, Jones CE (eds): MTP International Review of Science. Cardiovascular Physiology. Baltimore, University Park Press, 1974, p 163

71. Thomson JM, Dempsey JA, Chosy LW, et al: Oxygen transport and oxyhemoglobin dissociation during prolonged muscular work. J Appl Physiol 37:658, 1974

72. Keul J, Doll E: Intermittent exercise: metabolites, P_{O_2}, and acid-base equilibrium in the blood. J Appl Physiol 34:220, 1973

73. Mellander S, Lundvall J: Role of tissue hyperosmolarity in exercise hyperemia. Circ Res 28(Suppl 1):39, 1971

74. Kjellmer I: The potassium ion as a vasodilator during muscular exercise. Acta Physiol Scand 63:460, 1965

75. Skinner NS Jr, Costin JC: Interactions between oxygen, potassium, and osmolality in regulation of skeletal muscle blood flow. Circ Res 28(Suppl 1):73, 1971

76. Berne RM, Rubio R, Dobson JG Jr, et al: Adenosine and adenine nucleotides as possible mediators of cardiac and skeletal muscle blood flow regulation. Circ Res 28(Suppl 1):115, 1971

77. Scheuer J, Penpargkul S, Bhan AK: Experimental observations on the effects of physical training upon intrinsic cardiac physiology and biochemistry. Am J Cardiol 33:744, 1974

78. Pernow B, Saltin B: Muscle Metabolism During Exercise. Advances in Experimental Medicine and Biology, vol 2. New York, Plenum Press, 1971

79. Scheuer J: Physical training and intrinsic cardiac adaptations. Circulation 47:677, 1973

80. Vatner SF, Higgins CB, White S, et al: The peripheral vascular response to severe exercise in un-

tethered dogs before and after complete heart block. J Clin Invest 50:1950, 1971

81. Blair DA, Glover WE, Roddie IC: Vasomotor responses in the human arm during leg exercise. Circ Res 9:264, 1961

82. Vatner SF, Franklin D, Van Citters RL, et al: Effects of carotid sinus nerve stimulation on blood-flow distribution in conscious dogs at rest and during exercise. Circ Res 27:495, 1970

83. Donald DE, Rowland DJ, Ferguson DA: Similarity of blood flow in the normal and the sympathectomized dog hindlimb during graded exercise. Circ Res 26:185, 1970

84. Collier W: Functional albuminuria in athletes. Br Med J 1:4, 1907

85. MacKeith NW, Pembrey MS, Spurrell WR, et al: Observations on the adjustment of the human body to muscular work. Proc R Soc Lond (Biol) 95:413, 1923

86. Barcroft J, Harris HA, Orahovats D, et al: A contribution to the physiology of the spleen. J Physiol (Lond) 60:443, 1925

87. Barcroft J, Florey H: The effects of exercise on the vascular conditions in the spleen and colon. J Physiol (Lond) 68:181, 1929

88. Krogh A: The regulation of the supply of blood to the right heart with a description of a new circulation model. Scand Arch Physiol 27:227, 1912

89. Green HD, Hoff EC: Effects of faradic stimulation of the cerebral cortex on limb and renal volumes in the cat and monkey. Am J Physiol 118:641, 1937

90. Wexler I, Kao FK: Neural and humoral factors affecting canine renal blood flow during induced muscular work. Am J Physiol 218:755, 1970

91. Rowell LB: Human cardiovascular adjustments to exercise and thermal stress. Physiol Rev 54:75, 1974

92. Chapman CB, Henschel A, Minckler J, et al: The effect of exercise on renal plasma flow in normal male subjects. J Clin Invest 27:639, 1948

93. Bucht H, Ek J, Eliasch H, et al: The effect of exercise in the recumbent position on the renal circulation and sodium excretion in normal individuals. Acta Physiol Scand 28:95, 1953

94. Wade OL, Combes B, Childs AW, et al: The effect of exercise on the splanchnic blood flow and splanchnic blood volume in normal man. Clin Sci 15:457, 1956

95. Rowell LB, Blackmon JR, Bruce RA: Indocyanine green clearance and estimated hepatic blood flow during mild to maximal exercise in upright man. J Clin Invest 43:1677, 1964

96. Herrick JF, Grindlay JH, Baldes EJ, et al: Effect of exercise on the blood flow in the superior mesenteric, renal and common iliac arteries. Am J Physiol 128:338, 1939

97. Rushmer RF, Franklin DL, Van Citters RL, et al: Changes in peripheral blood flow distribution in healthy dogs. Circ Res 9:675, 1961

98. Vatner SF, Higgins CB, Millard RW, et al: Role of the spleen in the peripheral vascular response to severe exercise in untethered dogs. Cardiovasc Res 8:276, 1974

99. Higgins CB, Vatner SF, Franklin D, et al: Effects of experimentally produced heart failure on the peripheral vascular response to severe exercise in conscious dogs. Circ Res 31:186, 1972

100. Millard RW, Higgins CB, Franklin D, et al: Regulation of the renal circulation during severe exercise in normal dogs and dogs with experimental heart failure. Circ Res 31:881, 1972

101. Vatner SF, Higgins CB, Franklin D: Regional circulatory adjustments to moderate and severe chronic anemia in conscious dogs at rest and during exercise. Circ Res 30:731, 1972

102. Selkurt EE: The renal circulation, in Hamilton WF, Dow P (eds): Handbook of Physiology, sec 2, vol 2, Circulation. Baltimore, Williams & Wilkins, 1963, p 1457

103. Vane JR: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature (New Biol) 231:232, 1971

104. McGiff JC, Terragno NA, Malik KU, et al: Release of a prostaglandin E-like substance from canine kidney by bradykinin: Comparison with eledoisin. Circ Res 31:36, 1972

105. Vatner SF: Effects of hemorrhage on regional blood flow distribution in dogs and primates. J Clin Invest 54:225, 1974

106. Vatner SF, Patrick TA: Radiotelemetry of blood flow and pressure measurements in untethered conscious animals. Bibl Cardiol 34:1, 1974

Reprinted from Circulation Research
Vol. 39, No. 5, November 1976

The Role of Arterial Baroreceptors in the Regulation of Arterial Pressure in Conscious Dogs

ROBERT J. MCRITCHIE, M.B., STEPHEN F. VATNER, M.D., GUY R. HEYNDRICKX, M.D., AND EUGENE BRAUNWALD, M.D.

SUMMARY To elucidate the role of arterial baroreceptors in the acute regulation of arterial pressure in the conscious animal, arterial return to control levels, as well as the absolute change in arterial pressure, was considered (the pressure-time product), responses of

Reprinted from Circulation Research
Vol. 39, No. 5, November 1976

The Role of Arterial Baroreceptors in the Regulation of Arterial Pressure in Conscious Dogs

ROBERT J. MCRITCHIE, M.B., STEPHEN F. VATNER, M.D., GUY R. HEYNDRICKX, M.D., AND EUGENE BRAUNWALD, M.D.

SUMMARY To elucidate the role of arterial baroreceptors in the acute regulation of arterial pressure in the conscious animal, arterial pressure was lowered and raised in intact, conscious dogs, and in dogs after bilateral section of both carotid sinus and aortic nerves (total arterial baroreceptor denervation, TABD). Pressure was altered by intravenous bolus injections and continuous infusions of nitroglycerin and methoxamine and also by hemorrhage. TABD resulted in a change in peak mean arterial pressure 2–4 times as great as that seen in intact dogs following injection of nitroglycerin or methoxamine. However, when the time taken for the arterial pressure disturbance to return to control levels, as well as the absolute change in arterial pressure, was considered (the pressure-time product), responses of dogs with TABD were far greater for nitroglycerin (7–9 times that seen in intact dogs) and methoxamine (11–15 times). Arterial pressure responses of dogs with selective section of the carotid sinus nerves were intermediate but closer to those of intact dogs than to dogs with TABD. With infusion of drugs or following hemorrhage, responses of mean arterial pressure were 3- to 5-fold greater in dogs with TABD than in intact dogs, indicating that the static open loop gain of the arterial baroreceptor system ranged from 2 to 4.

THE ROLE of the major arterial baroreceptors in the regulation of arterial pressure has been a subject of considerable investigation.[1-14] Most studies delineating the function of these receptors have been performed in a variety of animal species and preparations, the majority of which have been anesthetized. However, since general anesthesia is now known to affect many aspects of circulatory control,[15] including the function of the baroreceptors,[16, 17] there is increasing interest in the role of these receptors in the conscious state. Accordingly, more recent studies have been performed in conscious dogs with denervated baroreceptors.[1, 2, 4, 10] A recent study by Cowley et al.[1] demonstrated that the arterial baroreceptor reflexes were not of importance in long-term regulation of arterial pressure.

The goal of the present investigation was to examine the role of the carotid sinus and aortic nerves in regulation of arterial pressure in response to acute hypotension and hypertension induced by alterations in peripheral vascular resistance, accomplished by injecting nitroglycerin and methoxamine intravenously. These drugs have little direct effect on the heart or central nervous system, but primarily affect the vascular bed to lower and raise arterial pressure.[18] In addition, the acute response to hypotension induced by rapid hemorrhage was examined. The role of the arterial baroreceptors was assessed by comparing the responses of arterial pressure and heart rate before and after recovery from bilateral denervation of both the aortic and carotid sinus nerves, i.e., total arterial baroreceptor denervation (TABD), and after recovery from denervation of only the carotid sinus nerves, leaving the aortic nerves intact.

From the Departments of Medicine, Harvard Medical School and Peter Bent Brigham Hospital, and Department of Cardiology, Childrens Hospital Medical Center, Boston, Massachusetts, and the New England Regional Primate Research Center, Southboro, Massachusetts.

Supported in part by U.S. Public Health Service Grants HL 15416, 17459, and HL 10436 and National Aeronautics and Space Administration-Ames Grant NSG 2136.

Dr. McRitchie was an Overseas Research Fellow of the National Heart Foundation of Australia, Dr. Vatner is an Established Investigator of the American Heart Association, and Dr. Heyndrickx was a U.S. Public Health Service International Research Fellow.

Address for reprints: Stephen F. Vatner, M.D., New England Regional Primate Research Center, 1 Pine Hill Drive, Southboro, Massachussetts 01772.

Received May 10, 1976; accepted for publication July 1, 1976.

Methods

A midline cervical incision was made and the carotid sinus nerves were sectioned bilaterally in seven dogs under pentobarbital Na anesthesia, 30 mg/kg. In 25 dogs both the carotid sinus and aortic nerves were sectioned bilaterally (TABD). Adequacy of carotid sinus nerve section was confirmed in all dogs at operation and in five dogs 1–2 weeks later by observing no change in arterial pressure or heart rate to bilateral carotid artery occlusion. The aortic nerves were sectioned according to the technique described by Edis and Shepherd.[3] The completeness of TABD was confirmed at operation and also in the conscious dogs on the day of study by observing the reflex heart rate responses to an intravenous bolus of nitroglycerin (nitroglycerin USP, Lilly), 48 μg/kg. Absence of heart rate changes following nitroglycerin and methoxamine was accepted as confirmation of TABD. Any dog showing a reflex heart rate response of more than 6 beats/min in a direction opposite to that of arterial pressure was excluded from the study. This resulted in excluding 13 of 25 dogs in which TABD was attempted.

Arterial pressure was sampled through the catheter previously implanted in the aorta and measured with a calibrated Statham P23Db strain gauge manometer. A cardiotachometer, triggered by the signal from the arterial pressure pulse, provided instantaneous and continuous records of heart rate. Data were recorded on a multichannel tape recorder and played back on a direct-writing oscillograph at a paper speed of 1 mm/sec. Statistical analysis was performed on the data according to standard techniques.[19]

Eighteen dogs were studied in the intact conscious state and 12 dogs 2–4 weeks following TABD. Five of these 12

dogs were studied both before and following denervation. All studies were carried out with the unsedated, trained dogs lying quietly in a darkened laboratory in order to avoid the wide fluctuations in arterial pressure that occur in denervated dogs if they are disturbed or excited.[1, 4]

Nitroglycerin and methoxamine were administered intravenously in bolus doses of 2, 4, 12, 24, and 48 μg/kg in a volume of 1 ml through an indwelling catheter. In addition, five intact dogs were infused with nitroglycerin (4, 8, 16, and 32 μg/kg per min) and methoxamine (8, 16, and 32 μg/kg per min), allowing 120 seconds for stabilization at each dose level. In addition to peak changes in mean arterial pressure and heart rate produced by bolus doses of the drugs, the total magnitude of the deviation in mean arterial pressure from control was measured (Fig. 1) by integrating it over the time taken for pressure to return to the control level by planimetry; this product is referred to as the "pressure-time product" and is expressed in mm Hg sec. On separate days hemorrhage at a rate of 1 ml/sec was induced until 25 ml/kg of blood were removed. To assess gain of arterial baroreceptors, the formula described by Milhorn[11] was used, i.e., open loop gain = [(ΔC) open/(ΔC) closed] − 1, where ΔC designates the steady state disturbance in arterial pressure induced by either nitroglycerin or methoxamine infusion or by hemorrhage, when the arterial baroreceptor reflex loop was open (TABD) or closed (intact).

Results

The control arterial pressures both in the dogs with carotid sinus nerve section alone (107 ± 5 mm Hg) and in those with TABD (111 ± 5 mm Hg) were significantly higher ($P < 0.05$) than in the intact group (92 ± 3 mm Hg). Heart rates of the intact dogs (83 ± 3 beats/min) and the dogs with carotid sinus nerve section (84 ± 6 beats/min) were similar and were significantly lower than those in the TABD group (115 ± 7 beats/min) ($P < 0.01$).

EFFECTS OF AORTIC AND CAROTID SINUS NERVE SECTION (TABD)

Nitroglycerin

In intact dogs nitroglycerin reduced mean arterial pressure and increased heart rate transiently. After recovery from TABD the same doses of nitroglycerin elicited no change in heart rate but 2- to 3-fold greater reductions in mean arterial pressure. In addition, the recovery time for arterial pressure was significantly longer (Fig. 1). The pressure-time products for all but the lowest dose of nitroglycerin were 7 to 9 times greater than normal (Table 1).

When nitroglycerin was administered by infusion to conscious, intact dogs, mean arterial pressure did not fall significantly at the lowest dose. At the larger doses approxi-

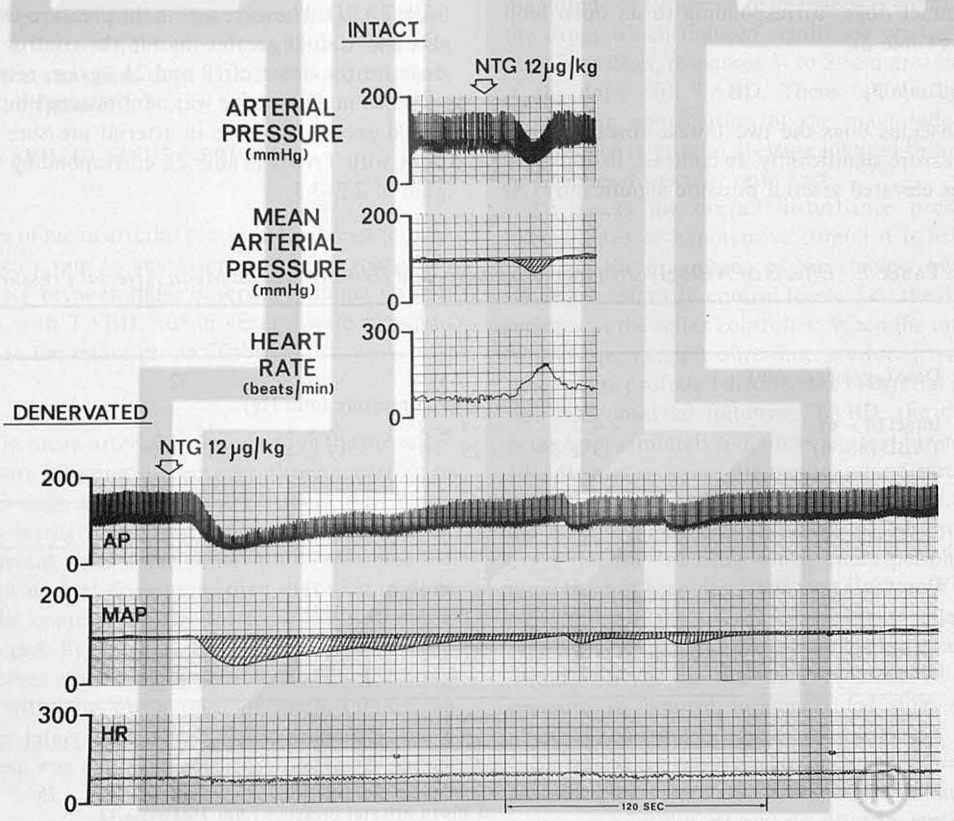


FIGURE 1 *Representative records of phasic and mean arterial pressure and heart rate in response to nitroglycerin (NTG), 12 μg/kg, in the intact state (above) and following TABD (denervated) in the same dog. Note, following denervation, the greater fall in pressure, the greatly prolonged recovery period, and the absence of a change in heart rate. The shaded area under the mean arterial pressure in the two records represents the area measured for determination of the pressure-time product.*

TABLE 1 *Effects of Nitroglycerin*

	Dose (μg/kg)	Intact (n = 18)	CSN section (n = 7)	TABD (n = 12)
Δ mean arterial pressure (mm Hg)	2	−12 ± 2	−17 ± 2	−28 ± 3*†
	4	−17 ± 1	−21 ± 2	−36 ± 3*†
	12	−22 ± 2	−29 ± 3‡	−51 ± 4*†
	24	−25 ± 2	−33 ± 2*	−53 ± 4*†
	48	−26 ± 1	−34 ± 2*	−59 ± 3*†
Pressure-time product (mm Hg sec)	2	−124 ± 24	−236 ± 88	−1496 ± 288*†
	4	−228 ± 24	−376 ± 80‡	−1660 ± 264*†
	12	−392 ± 32	−676 ± 100*	−3224 ± 684*†
	24	−604 ± 52	−1120 ± 152*	−4336 ± 808*†
	48	−732 ± 72	−1192 ± 232‡	−6568 ± 1072*†
Δ heart rate (beats/min)	2	32 ± 4	14 ± 3‡	−1 ± 2*†
	4	49 ± 4	26 ± 6*	+2 ± 1*†
	12	69 ± 4	37 ± 5‡	+1 ± 1*†
	24	77 ± 5	49 ± 7‡	+2 ± 1*†
	48	83 ± 6	48 ± 7*	+1 ± 1*†

CSN = carotid sinus nerve; TABD = total arterial baroceptor denervation; n = number of dogs.
* Significantly different from intact ($P < 0.01$).
† TABD significantly different from carotid sinus nerve section ($P < 0.01$).
‡ Significantly different from intact ($P < 0.05$).

mately 3- to 5-fold greater reductions in mean arterial pressure occurred in the dogs with TABD as compared to the conscious intact dogs, corresponding to an open loop gain of 2.5–3.8 (Table 2).

Methoxamine (*Table 3*)

In intact, conscious dogs the two lowest doses failed to alter arterial pressure significantly. In contrast, in dogs with TABD all doses elevated arterial pressure significantly. At the three highest doses, methoxamine induced a 2- to 4-fold greater increase in mean arterial pressure in conscious dogs with TABD. The increases in the pressure-time product were 11- and 15-fold greater than in the control intact conscious dogs for the doses of 12 and 24 μg/kg, respectively.

When methoxamine was administered by infusion, a 3- to 5-fold greater increase in arterial pressure occurred in the dogs with TABD (Table 2), corresponding to an open loop gain of 2.2–3.5.

TABLE 2 *Effects of Nitroglycerin, Methoxamine, and Hemorrhage on Mean Arterial Pressure*

Nitroglycerin infusion					
Dose (μg/kg per min)	4	8	16	32	
	Δ mean arterial pressure (mm Hg)				
Intact (n = 6)	−4 ± 1	−7 ± 2	−9 ± 2	−13 ± 2	
TABD (n = 6)	−19 ± 2*	−29 ± 3*	−39 ± 4*	−45 ± 3*	
Gain	3.8	3.1	3.3	2.5	
Methoxamine infusion					
Dose (μg/kg per min)	8	16	32		
	Δ mean arterial pressure (mm Hg)				
Intact (n = 6)	4 ± 1	8 ± 2	20 ± 3		
TABD (n = 6)	18 ± 5*	41 ± 7*	64 ± 9*		
Gain	3.5	4.1	2.2		
Hemorrhage					
Blood loss (ml/kg)	5	10	15	20	25
	Δ mean arterial pressure (mm Hg)				
Intact (n = 12)	−5 ± 1	−8 ± 2	−10 ± 2	−13 ± 4	−20 ± 3
TABD (n = 12)	−12 ± 2*	−26 ± 4*	−37 ± 4*	−48 ± 4*	−60 ± 5*
Gain	1.4	2.3	2.7	2.7	2.0

TABD = total arterial baroreceptor denervation; n = number of dogs.
* TABD significantly different from intact ($P < 0.01$).

TABLE 3 *Effects of Methoxamine*

	Dose (μg/kg)	Intact (n = 18)	CSN section (n = 7)	TABD (n = 12)
Δ mean arterial pressure (mm Hg)	2	0.5 ± 0.7	1 ± 1	+7 ± 2*
	4	1.1 ± 0.3	7 ± 1*†	+12 ± 2*
	12	6 ± 1	10 ± 1*‡	+27 ± 2*
	24	10 ± 1	16 ± 1*‡	+35 ± 3*
	48	20 ± 3	27 ± 3§‡	+44 ± 7*
Pressure-time product (mm Hg sec)	2	8 ± 8	40 ± 28†	124 ± 24*
	4	28 ± 12	96 ± 12*†	1028 ± 380*
	12	584 ± 172	1848 ± 144*†	6204 ± 1960*
	24	1248 ± 180	3812 ± 772*‡	19,152 ± 3480*
	48	7200 ± 913	5744 ± 676‡	27,600 ± 3480*
Δ heart rate (beats/min)	2	0 ± 0	0 ± 0	−1 ± 1*
	4	−4 ± 1	−2 ± 2	−1 ± 1*
	12	−15 ± 4	−16 ± 2‡	−1 ± 1*
	24	−21 ± 2	−25 ± 5‡	−1 ± 1*
	48	−37 ± 3	−37 ± 6‡	−1 ± 1*

CSN = carotid sinus nerve; TABD = total arterial baroreceptor denervation; n = number of dogs.
* Significantly different from intact ($P < 0.01$).
† Carotid sinus nerve section significantly different from TABD ($P < 0.05$).
‡ Carotid sinus nerve section significantly different from TABD ($P < 0.01$).
§ Significantly different from intact ($P < 0.05$).

Hemorrhage (Table 2)

In intact, conscious dogs, hemorrhage reduced mean arterial pressure significantly only after 10 ml/kg of blood loss. With comparable blood loss hemorrhage induced approximately a 3- to 4-fold greater reduction in mean arterial pressure in conscious dogs with TABD, corresponding to an open loop gain of 2.0–2.7.

SECTION OF CAROTID SINUS NERVES

Nitroglycerin

The responses of mean arterial pressure, the pressure-time product, and heart rate to any given dose of nitroglycerin were intermediate between those observed in intact normal dogs and those with TABD, but in general were closer to those observed in the intact group (Table 1).

Methoxamine

The changes in mean arterial pressure and in the pressure-time product were intermediate between those occurring in intact conscious dogs and those with TABD (Table 3). As was the case for nitroglycerin, the response to methoxamine in dogs with carotid sinus nerve denervation resembled the response seen in normal dogs more than that seen in dogs with TABD. In contrast to the intermediate heart rate response produced by nitroglycerin, denervation of the carotid sinus nerves alone did not alter the extent of slowing of heart rate with any given dose of methoxamine, as compared to the intact dogs, although the pressor response to any given dose was augmented.

Discussion

The role of the arterial baroreceptors in the immediate, short-term regulation of arterial pressure has been investigated predominantly in anesthetized animals. In the present study, nitroglycerin and methoxamine were injected to determine the extent to which arterial baroreceptors buffer an acute change in arterial pressure in conscious dogs with arterial baroreceptors intact and sectioned. When only the magnitude of the pressure change was compared at doses of the drugs which induced significant changes in the intact, conscious dogs, responses 3- to 5-fold greater were observed in the dogs with TABD. These figures, however, do not provide an appreciation of the magnitude of the overall disturbance in arterial pressure induced by an abrupt change in peripheral vascular resistance.

To assess the overall disturbance produced by these hypertensive or hypotensive stimuli it is helpful to include not only the magnitude of the change, but also the time taken for return to control levels, i.e., the dynamic characteristics of the reflex controller. When the total disturbances in pressure, i.e., pressure-time products, were examined, a much more profound disturbance of arterial pressure regulation was observed following TABD, the disturbance now being approximately 6–8 times as great for nitroglycerin and 10–14 times as great for methoxamine as that observed in intact, conscious dogs. This type of analysis, which indicates the total disturbance induced by a fixed stimulus, i.e., an exact quantity of drug which alters peripheral vasomotor tone, has not been described previously, but underscores the importance of the arterial baroreceptor reflexes in the acute regulation of arterial pressure. Cowley et al.[1] also noted a 4-fold increase in the time required to return arterial pressure to control in denervated dogs in response to postural changes.

To assess the steady state open loop gain of the arterial baroreceptor system in the conscious animal, steady state responses of mean arterial pressure to continuous infusions of nitroglycerin and methoxamine, as well as to a physiological stimulus, i.e., hemorrhage, were compared in intact dogs and dogs with TABD. Using either the infusions of nitroglycerin and methoxamine or hemorrhage to disturb

pressure, the calculated open loop gain ranged from 2 to 4. Considering that the gain of the baroreceptor is a nonlinear function,[8, 9] it was surprising to note the consistency of values derived in these conscious dogs in response to both hypertension and hypotension. Although the reported values for gain vary considerably, the majority of other studies performed to assess the gain of the arterial baroreceptors have reported values between 1 and 2.[1, 8, 11, 13] Exceptions are the study by Scher and Young,[9] in which gain varied considerably, and a preliminary report by Brown and Taylor,[20] in which acute adjustments to sinusoidal alterations in blood volume were examined in dogs with and without arterial baroreceptor nerves. It is interesting that the latter study also was conducted in unanesthetized dogs.[20] The difference between our findings and previous studies may be explained, in part, by differing definitions of the term "gain," as well as by differences of the experimental preparations, making precise comparisons of the gain derived from other studies with those from the present investigation difficult. Moreover, in the present investigation both the carotid sinus and aortic afferents were eliminated in the conscious dog, whereas most previous studies concentrated on the carotid sinus reflex in anesthetized animals.[9, 11, 13] Cowley et al.[1] also used conscious dogs and did not report as high a value for gain as we did. However, in that investigation gain was assessed from 24-hour arterial pressure distribution curves as opposed to only acute quantifiable interventions, which were examined in the present study.

The technique of TABD used in this study, i.e., bilateral cervical section of the carotid sinus and aortic nerves leaving the vagi intact,[3] was selected because it left other vagal and sympathetic afferents intact. On the other hand, it is recognized that it could result in incomplete denervation to the extent that some aortic baroreceptor fibers traverse the cervical vagus nerves of the dog.[6, 7] Accordingly, 13 dogs that did exhibit small reciprocal changes in heart rate in response to hypertensive stimuli following the denervation procedure were excluded from this study in the belief that they had incomplete section of the aortic nerves. The 12 dogs that were studied did not demonstrate reciprocal heart rate changes in response to an alteration in arterial pressure. Thus, if remaining aortic baroreceptor fibers traveling in the vagi did play a role in regulation of arterial pressure in these experiments, their functional significance was minor insofar as their ability to induce reflex heart rate responses is concerned.

A recent study conducted in anesthetized dogs has shown that the aortic, unlike the carotid, baroreceptors are relatively ineffective in buffering a reduction in systemic arterial pressure below normal levels, compared to their greater potency in buffering elevations of arterial pressure.[12] One of the conclusions drawn from that investigation was that the aortic baroreceptors act primarily to prevent acute hypertension,[12] whereas the carotid sinus nerves buffer both hypertensive and hypotensive stimuli. To examine the contribution to arterial pressure regulation of the aortic receptors alone, we studied a series of conscious dogs after their recovery from bilateral section of the carotid sinus nerves, with the aortic nerves left intact. In this group of dogs the perturbations of arterial pressure were significantly greater with both hypotensive and hypertensive stimuli than they were in intact dogs, but far less than were observed in dogs with TABD. From these data, it is clear that the aortic nerves alone can act as an effective mechanism to buffer both elevations and reductions in arterial pressure. Thus, the results of the present study suggest that in conscious dogs aortic baroreceptors alone are able to buffer reductions as well as increases in arterial pressure.

In conclusion, this study has demonstrated the extent to which carotid sinus and aortic arch arterial baroreceptors buffer arterial pressure in response to acute hypotension or hypertension in the conscious dog. Removing arterial baroreceptors altered the time required for arterial pressure to return to baseline as much as it altered the magnitude of the pressure response to a hyper- or hypotensive agent. The aortic arch baroreceptors appeared important in buffering hypotensive as well as hypertensive responses. Values for static open loop gain of the combined baroreceptor systems in the conscious dog ranged from 2 to 4.

References

1. Cowley AW, Liard JF, Guyton AC: Role of the baroreceptor reflex in daily control of arterial blood pressure and other variables in dogs. Circ Res **32:** 564–576, 1973
2. Cowley AW, Monos E, Guyton AC: Interaction of vasopressin and the baroreceptor reflex system in the regulation of arterial blood pressure in the dog. Circ Res **34:** 505–514, 1974
3. Edis AJ, Shepherd JT: Selective denervation of aortic arch baroreceptors and chemoreceptors in dogs. J Appl Physiol **30:** 294–296, 1971
4. Ferrario CM, McCubbin JW, Page IH: Hemodynamic characteristics of chronic experimental neurogenic hypertension in unanesthetized dogs. Circ Res **24:** 911–922, 1969
5. Heymans C, Neil E: Reflexogenic Areas of the Cardiovascular System. Boston, Little, Brown, 1958
6. Ito CS, Scher AM: Arterial baroreceptor fibers from the aortic region of the dog in the cervical vagus nerve. Circ Res **32:** 442–446, 1973
7. Ito CS, Scher AM: Reflexes from the aortic baroreceptor fibers in the cervical vagus of the cat and dog. Circ Res **34:** 51–60, 1974
8. Korner PI: Integrative neural cardiovascular control. Physiol Rev **51:** 312–367, 1971
9. Scher AM, Young AC: Servoanalysis of carotid sinus reflex effects on peripheral resistance. Circ Res **12:** 152–162, 1963
10. Krasney JA, Magno MG, Levitsky MG, Koehler RC, Davies DG: Cardiovascular responses to arterial hypoxia in awake sinoartic-denervated dogs. J Appl Physiol **35:** 73–738, 1973
11. Milhorn HT: The Application of Control Theory to Physiological Systems. Philadelphia, Saunders, 1966
12. Pelletier CL, Shepherd JT: Circulatory reflexes from mechanoreceptors in the cardio-aortic area. Circ Res **33:** 131–138, 1973
13. Sagawa K: Relative roles of the rate sensitive and proportional control elements of the carotid sinus during mild hemorrhage. *In* Baroreceptors and Hypertension, edited by P Kezdi. New York, Pergamon Press, 1967
14. Kumada M, Schmidt RM, Sagawa K, Tan KS: Carotid sinus reflex in response to hemorrhage. Am J Physiol **219:** 1373–1379, 1970
15. Vatner SF, Braunwald E: Cardiovascular control mechanisms in the conscious state; a comparison of the effects of physiological and pharmacological stimuli in the presence and absence of general anesthesia. N Engl J Med **293:** 970–976, 1975
16. Vatner SF, Franklin D, Braunwald E: Effects of anesthesia and sleep on circulatory response to carotid sinus nerve stimulation. Am J Physiol **220:** 1249–1255, 1971
17. Vatner SF, Higgins CB, Franklin D, Braunwald E: Extent of carotid sinus regulation of the myocardial contractile state in conscious dogs. J Clin Invest **51:** 995–1008, 1972
18. Goodman LS, Gilman A: The Pharmacologic Basis of Therapeutics, ed 4. New York, MacMillan, 1970
19. Snedecor GW, Cochran WG: Statistical Methods, ed 6. Ames, Iowa State University Press, 1967
20. Brown DR, Taylor AE: Arterial pressure response to sinusoidal blood volume changes in unanesthetized control and sino-aortic denervated dogs (abstr). Fed Proc **31:** 367, 1972

Effects of Propranolol on Regional Myocardial Function, Electrograms, and Blood Flow in Conscious Dogs with Myocardial Ischemia

STEPHEN F. VATNER, HANK BAIG, W. THOMAS MANDERS, HERMANN OCHS, and MASSIMO PAGANI

From the Departments of Medicine, Harvard Medical School and Peter Bent Brigham Hospital, the Department of Cardiology, Children's Hospital Medical Center, Boston, Massachusetts 02115 and the New England Regional Primate Research Center, Southboro, Massachusetts 01772

ABSTRACT The effects of coronary occlusion and of subsequent propranolol administration were examined in 18 conscious dogs. Overall left ventricular (LV) function was assessed by measurements of LV pressure and dP/dt, and regional myocardial function was assessed by measurements of segment length (SL), velocity of SL shortening and regional myocardial "work", i.e., pressure-length loops in normal, moderately, and severely ischemic zones. Regional intramyocardial electrograms were measured from the same sites along with regional myocardial blood flow as determined by the radioactive microsphere technique. Coronary occlusion resulted in graded loss of function from the normal to severely ischemic zones with graded flow reduction and graded elevation of the ST segment. Propranolol depressed overall LV function, function in the normal zone (work fell by 17±4%), and in the majority of moderately ischemic segments (work fell by 7±3%). In severely ischemic segments the extent of paradoxical motion and post-systolic shortening was reduced by propranolol. After propranolol regional myocardial blood flow fell in the normal zone (11±2%) and rose in the moderately (15±4%) and severely (63±10%) ischemic zones. Thus, in the conscious dog with regional myocardial ischemia, propranolol induces a redistribution of myocardial blood flow, with flow falling in normal zones and rising in moderately and severely ischemic zones. The improvement in perfusion of ischemic tissue was associated with slight but significant depression of shortening, velocity, and work in the moderately ischemic zones and of paradoxical bulging and post-systolic shortening in the severely ischemic zone.

INTRODUCTION

Propranolol has been shown to reduce experimental infarct size after coronary occlusion in anesthetized animal preparations (1–3). Two possible mechanisms, which would result in protection of ischemic myocardium, involve an increase oxygen supply, i.e., in blood flow, or a reduction in oxygen demands, i.e., the work of the ischemic tissue. Prior studies in anesthetized animals have consistently shown no effect of propranolol on blood flow to ischemic myocardium (4–6), while previous studies on the effects of beta adrenergic blockers on function of ischemic tissue have been controversial. On the one hand propranolol has been shown to depress overall left ventricular function (7–9) and regional function (10) of the ischemic heart, while on the other hand beta adrenergic blockers have also been shown to improve regional function in the presence of ischemia (11, 12).

While prior studies have examined the effects of beta adrenergic blockers on measurements of electrograms (1–3, 5, 10, 13) mechanical function (10–12), and regional myocardial blood flow (4–6) in the ischemic heart, these measurements have not been correlated in the same study. Moreover, most of these previous studies have been conducted in anesthetized animals with an open chest (1–6, 10, 12, 13), where the myocardial depressant effects of the anesthesia (14, 15) and recent surgery could intensify the depressant effects of the beta adrenergic blocker. It is also important to keep in mind that in the presence of regional myocardial ischemia, measurements of overall function can be misleading, since function can be entirely normal in one portion of the heart and absent in another. Accordingly, this investigation

Dr. Vatner is an Established Investigator of the American Heart Association. Dr. Pagani is a Postdoctoral Research Fellow, 1 F05 TW02331-01, of the U. S. Public Health Service.

Received for publication 31 December 1976 and in revised form 14 March 1977.

was conducted in conscious dogs, in which the effects of propranolol were examined with simultaneous measurements of regional myocardial function, blood flow, and electrograms in normal, moderately, and severely ischemic zones. The specific goals of this study were to ascertain (*a*) whether function of ischemic myocardium improved or deteriorated with propranolol and (*b*) whether in the conscious dog, the change in function was associated with an alteration in blood flow to the ischemic myocardium.

METHODS

30 dogs, weighing between 25 and 35 kg, were anesthetized with i.v. pentobarbital sodium, 30 mg/kg. Through a thoracotomy in the fifth left intercostal space, miniature pressure gauges (P_{22}, Konigsberg Instruments, Inc., Pasadena, Calif.) were implanted within the left ventricle through a stab wound in the apex, and Doppler ultrasonic flow transducers were placed around either the left anterior descending (18 dogs) or circumflex coronary arteries (12 dogs), 2–3 cm from the bifurcation of these vessels. Hydraulic occluders were implanted just distal to the flow transducers and heparin-filled Tygon catheters (Norton Co., Plastics & Synthetics Div., Akron, Ohio) were implanted in the left atrium and aorta. Up to six pairs of miniature ultrasonic transducers[1] were implanted intramyocardially, parallel to the muscle fibers, 1–2 cm apart and varying in depth from 4 to 15 mm, in potentially normal, moderately, and severely ischemic zones.

The miniature pressure gauges were calibrated in vitro and in vivo against a calibrated Statham P23 Db strain gauge manometer (Statham Instruments Div., Gould, Inc., Oxnard, Calif.) connected to the left atrial and aortic catheters. At autopsy the position of the gauges within the ventricular cavity was confirmed. Instantaneous coronary blood flow was measured with an ultrasonic Doppler flowmeter (16, 17). An improved ultrasonic transit-time dimension gauge was used to measure regional myocardial segment length (SL)[2] (11, 18, 19). The instrument generates a voltage linearly proportional to the transit time of acoustic impulses traveling at the sonic velocity of approximately 1.5×10^6 mm/μs between the 3 MHz piezoelectric crystals, thus giving a record of instantaneous myocardial fiber length. At a constant room temperature the thermal drift of the instrument is minimal, i.e., less than 0.01 mm in 6 h. The frequency response is flat to 60 Hz. Any drifts in the measuring system, i.e., in the instrument electronics, the data tape recorder, and the oscillograph that displayed data, were eliminated during the experiment by periodic calibrations. This involved substitution of pulses of precisely known duration from a crystal-controlled pulse generator having a basic stability of 0.001%. The instrument used in the present study was modified further to provide simultaneous measurement of eight segment lengths and the regional electrogram from all crystal sites, located in normal, border, and ischemic zones. The position of the miniature ultrasonic transducers was confirmed at autopsy and minimal fibrosis, less than 1 mm, was observed at the site of implantation.

Regional myocardial blood flow was measured by the radioactive microsphere technique (20). The microspheres (3M Co., St. Paul, Minn.) were suspended in 0.01% Tween[80] solution (10% dextran) and placed in an ultrasonic bath for 60 min. They were subsequently agitated by direct application of an ultrasonic probe to insure dispersion of the spheres just before injection. Absence of microsphere aggregation was verified by microscopic examination. Before injection of microspheres, 0.7 ml of the Tween[80]—dextran solution (without microspheres) was injected to determine if the diluent for the microsphere suspension was to have an adverse effect on cardiac dynamics (21). Four to six million microspheres (9±2 μm) labeled with ^{46}Sc, ^{51}Cr, ^{85}Sr, or ^{141}Ce and suspended in 10% dextran, were injected through the catheter implanted in the left atrium for three determinations of blood flow; during control, then 10–15 min after the onset of coronary occlusion, and finally 5–20 min after propranolol. A reference sample of arterial blood was withdrawn beginning 10 s before microsphere injection and continuing for 40 s after the injection was completed. After sacrifice of the animal, myocardial samples were obtained from the sites where function and electrograms were measured, dissected into epi- and endocardial layers, weighed, placed in a three-channel gamma well counter (Searle Analytic Inc., Des Plaines, Ill.), and counted in appropriately selected energy windows for 10 min. The raw counts were then corrected for background and cross-over and compared with the reference blood sample to obtain flow expressed in milliliters per minute per gram of tissue.

Experiments were conducted 2–4 weeks after operation. While the conscious, unsedated dogs rested quietly, control records of left ventricular (LV) pressure (P), the rate of change of pressure (dP/dt), coronary blood flow, heart rate, multiple segment lengths (SL), and velocity (V) of SL shortening were recorded, along with intramyocardial electrograms. After control measurements were recorded, including the first injection of microspheres, the coronary vessel was occluded and occlusion was confirmed by absence of coronary flow until termination of the animal. Measurements were recorded continuously and the second microsphere injection was made 10–15 min after coronary occlusion, at a time when measurements of regional myocardial function and electrograms were stable. At 15–20 min after coronary occlusion propranolol was injected in doses of 0.5 mg/kg (3 dogs), 1.0 mg/kg (12 dogs), and 2.0 mg/kg (3 dogs). Qualitative differences in response were not observed among the three doses. The third microsphere injection was made 5–20 min after propranolol. After 30 min of further recordings the animals were anesthetized with 30 mg/kg of pentobarbital sodium and sacrificed to confirm placement of intramyocardial transducers and to obtain myocardial samples at the same sites for regional blood flow determination. 12 additional dogs (controls) were studied with similar protocols. In the six control dogs studied for measurement of regional blood flow, saline instead of propranolol was administered before the third microsphere injection.

Data were recorded on a multichannel tape recorder and played back on two multichannel direct-writing oscillographs at a paper speed of 100 mm/s. A cardiotachometer, triggered by the pressure pulse signal, provided instantaneous and continuous records of heart rate. Continuous records of dP/dt, and dSL/dt were derived from the signals of LVP and SL with Philbrick (Teledyne Philbrick, Dedham, Mass.) operational amplifiers connected as differentiators having frequency responses of 700 and 140 Hz, respectively. A triangular wave signal with known slope (rate of change) was substituted for P and SL signals to calibrate the differentiators directly.

The effects of interventions on regional myocardial function were assessed by measurement of stroke shortening,

[1] Construction details available from the authors.

[2] *Abbreviations used in this paper*: dP/dt, rate of change of pressure; ENDO-EPI, endocardial/epicardial flow ratio; LV, left ventricular; P, pressure; SL, segment length; V, velocity.

velocity of segment shortening, and end-diastolic and end-systolic segment lengths. In addition an *x-y* plot of the instantaneous LV pressure and regional SL signals were recorded and photographed from a storage oscilloscope. The area described by this loop was taken as an index of regional myocardial "work" in units of millimeters Hg–millimeter. End-diastolic length was the point just before isovolumetric contraction. End-systole coincided with isovolumetric relaxation. These points were readily identifiable in most instances. However, the precise timing of the end-systolic point may have varied by as much as 0.01 s, which could introduce a slight error in some ischemic segments.

Average and SEM values were calculated. The three states in each animal (control, occlusion, and occlusion plus propranolol) were compared by the paired *t* test, while changes between states were compared in the untreated controls and propranolol-treated animals by the unpaired *t* test (22).

RESULTS

Effects of coronary occlusion

Overall LV Function (n = 18) (Table I)

After coronary occlusion, heart rate rose by 31±5%, $P < 0.01$, from a control of 81±4 beats/min. LV systolic pressure and peak dP/dt did not change significantly, from control levels of 114±2 mm Hg and 3,330±140 mm Hg/s, respectively.

Regional LV Function (Figs. 1, 2; Table II)

Normal zone (19 segments). After coronary occlusion end-diastolic SL, SL stroke shortening, velocity, and work did not change significantly.

Table I

Overall LV Function: Effects of Coronary Occlusion and Subsequent Propranolol Administration (n = 18) Compared with Untreated Controls (n = 12)

	Preocclusion control	Occlusion	Occlusion and propranolol
LV systolic pressure, *mm Hg*			
Propranolol	114±1.5	115±2.9	116±3.1
Untreated	114±2.4	118±3.1	117±3.2
LV dP/dt, *mm Hg/s*			
Propranolol	3,330±140	3,240±150	2,700±110*‡§
Untreated	3,130±150	3,090±150	3,030±110
Heart rate, *beats/min*			
Propranolol	81±4.4	104±5.1*¶	96±3.9*‡¶
Untreated	80±3.3	116±3.3*	118±3.6

* Significantly different from preocclusion control, $P < 0.01$.
‡ Significantly different from occlusion value, $P < 0.01$.
§ Response of two groups significantly different, $P < 0.01$.
¶ Response of two groups significantly different, $P < 0.05$.

Moderately ischemic zone (29 segments). Coronary occlusion increased end-diastolic SL by 3.2±0.5% from a control of 17.6±1.0 mm and reduced SL stroke shortening by 59±4% from a control of 2.42±0.21 mm, velocity by 49±3% from a control of 24±2 mm/s, and work by 49±4%, from a control of 238±29 mm Hg–mm. All these changes were significant, $P < 0.01$.

Severely ischemic zone (45 segments). Coronary occlusion increased end-diastolic SL by 6.5±0.8%, from a control of 16.59±0.68 mm, and reduced stroke SL shortening by 116±4% from a control of 2.10±0.15 mm, velocity by 93±2% from a control of 23.3±1.7 mm/s, and "work" by 92±3% from a control of 229±19 mm Hg–mm. All these changes were significant, $P < 0.01$. Since the majority of these segments had larger end-systolic dimensions than end-diastolic dimensions (paradoxical motion), the reduction in SL stroke shortening was greater than 100%.

Intramyocardial Electrogram (Table III)

Coronary occlusion failed to elicit ST elevation in the normal zone, but increased ST elevation by 3.1 ±0.7 mV, $P < 0.01$, in the moderately ischemic zone

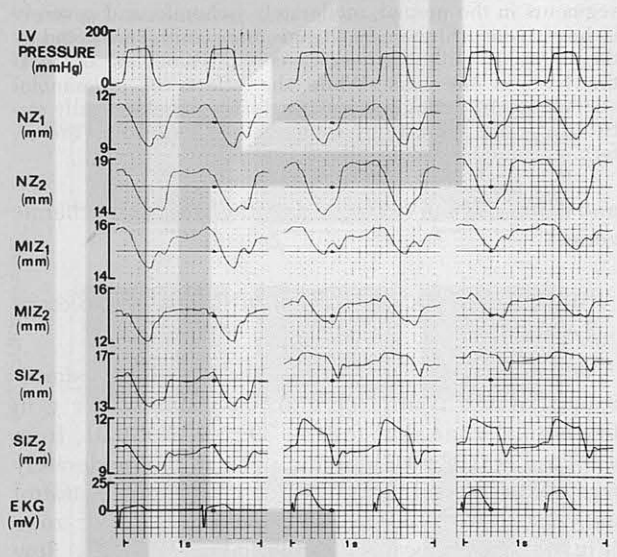


Figure 1 The simultaneous phasic wave forms at rapid paper speed are shown for left ventricular (LV) pressure, segment lengths in two normal zones (NZ), two moderately ischemic zones (MIZ), and two severely ischemic zones (SIZ), along with an electrogram from one of the severely ischemic segments during control (left panel), after coronary occlusion (middle panel), and after propranolol during coronary occlusion (right panel). With occlusion function fell slightly in one of the normal zones (NZ_1) and improved in the other (NZ_2). Function fell more strikingly in the moderately ischemic zones and was completely lost in the severely ischemic zones. Propranolol induced less dramatic effects, depressing function slightly in normal and moderately ischemic zones, and decreasing passive stretching in severely ischemic zones.

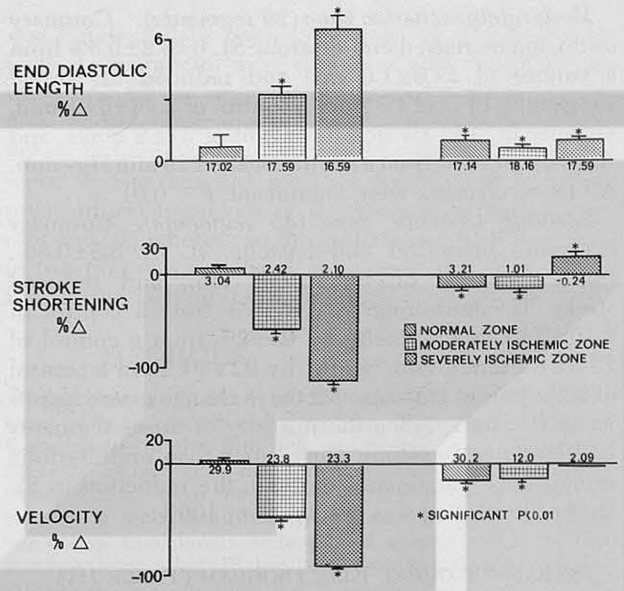


FIGURE 2 The effects of coronary occlusion (left panel) and of propranolol during occlusion (right panel) are shown as percentage change from control for end diastolic segment length, stroke shortening, and velocity of shortening for all segments in the normal, moderately ischemic, and severely ischemic zones. Significant changes from control are noted by the asterisks, while the average control values are noted at the base of the bars. While the effects of propranolol administration during coronary occlusion were generally statistically significant, they were small in relation to the effects induced by simple coronary occlusion.

and by 7.8±0.9 mV, $P < 0.01$ in the severely ischemic zone.

REGIONAL MYOCARDIAL BLOOD FLOW (FIG. 3, TABLE IV)

With coronary occlusion flow did not change significantly from a control of 1.10±0.03 ml/min per g in the normal zone, but fell by 39±3%, $P < 0.01$, from a control of 0.92±0.03 ml/min per g in the moderately ischemic zone and by 82±2%, $P < 0.01$, from a control of 0.98±0.04 ml/min g in the severely ischemic zone (Fig. 3). The endocardial/epicardial (ENDO/EPI) flow ratio did not change significantly in the normal zone (control = 1.19±0.03) but fell ($P < 0.01$) in the moderately ischemic zone from 1.22±0.05 to 0.82±0.06, and from 1.19±0.03 to 0.63±0.08 in the severely ischemic zone.

Effects of propranolol in the presence of coronary occlusion

OVERALL LV FUNCTION (TABLE I)

Propranolol did not affect LV systolic pressure significantly, but reduced heart rate by 6.4±1.9%, $P < 0.01$,

TABLE II

Regional Function: Effects of Coronary Occlusion and Subsequent Propranolol Administration (n = 18) Compared with Untreated Control Animals (n = 12)

	Preocclusion control	Occlusion	Occlusion and propranolol
Normal zone			
Stroke shortening, *mm*			
Propranolol	3.04±0.25	3.21±0.27	2.79±0.26*‡¶
Untreated	1.88±0.29	2.04±0.27	2.02±0.27
Velocity, *mm/s*			
Propranolol	29.9±2.65	30.2±2.77	25.1±2.11*‡§
Untreated	23.1±2.83	23.8±2.26	23.7±2.22
End diastolic length, *mm*			
Propranolol	17.02±1.09	17.14±1.13	17.28±1.14*‡§
Untreated	16.95±1.82	17.18±1.86*	17.15±1.88
Moderately ischemic zone			
Stroke shortening, *mm*			
Propranolol	2.42±0.21	1.01±0.12*	0.92±0.13*‡§
Untreated	1.93±0.29	0.88±0.17*	1.00±0.18*
Velocity, *mm/s*			
Propranolol	23.8±1.67	12.0±0.91*	11.0±1.02*‡§
Untreated	19.6±2.28	10.2±1.28*	11.1±1.24*
End diastolic length, *mm*			
Propranolol	17.59±0.99	18.16±1.03*	18.47±1.06*‡¶
Untreated	13.85±1.08	14.24±1.18*	14.21±1.18*
Severely ischemic zone			
Stroke shortening, *mm*			
Propranolol	2.10±0.15	−0.24±0.09*	−0.17±0.08*‡¶
Untreated	1.95±0.29	−0.26±0.09*	−0.28±0.12*
Velocity, *mm/s*			
Propranolol	23.3±1.69	2.09±0.80*	1.74±0.76*
Untreated	22.0±2.78	0.30±0.30*	1.25±0.71*
End diastolic length, *mm*			
Propranolol	16.59±0.68	17.59±0.70*	17.77±0.71*‡§
Untreated	14.85±0.77	15.36±0.75*	15.32±0.75*

* Significantly different from preocclusion control, $P < 0.01$.
‡ Significantly different from occlusion value, $P < 0.01$.
§ Response of two groups significantly different, $P < 0.01$.
¶ Response of two groups significantly different, $P < 0.05$.

TABLE III

Regional Electrocardiogram: Effects of Coronary Occlusion and Subsequent Propranolol Administration (n = 18) Compared with Untreated Controls (n = 10)

	Preocclusion control	Occlusion	Occlusion and propranolol
		mV	
Normal zone			
Propranolol	0.7±0.14	0.7±0.13	0.7±0.16
Untreated	0.8±0.25	0.6±0.27	0.7±0.29
Moderately ischemic zone			
Propranolol	0.9±0.12	4.0±0.71*	3.9±0.72*
Untreated	0.7±0.28	4.3±0.93*	4.0±0.85*
Severely ischemic zone			
Propranolol	0.6±0.09	8.5±0.93*	8.7±0.91*
Untreated	0.5±0.16	6.9±0.87*	6.7±0.87*

* Significant change from preocclusion control, $P < 0.01$.

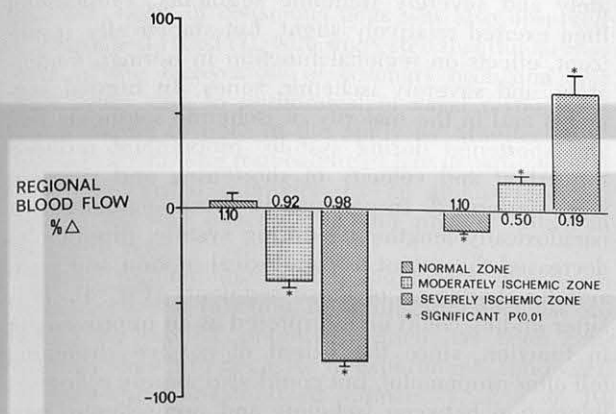


FIGURE 3 The effects of coronary occlusion (left) and subsequent propranolol during coronary occlusion (right) on regional myocardial blood flow are shown as percentage change from control for all segments studied in the normal, moderately ischemic, and severely ischemic zones. Significant changes from control are denoted by the asterisks while control values are noted at the base of the bars. Propranolol induced a significant redistribution of myocardial blood flow with flow falling in the normal zone and rising in the moderately and severely ischemic zones.

from an occlusion control of 104±5 beats/min and dP/dt by 15.4±2.1%, $P < 0.01$, from an occlusion control of 3,240±150 mm Hg/s.

REGIONAL LV FUNCTION (FIGS. 1, 2, TABLE II)

Normal zone. Propranolol increased end-diastolic SL by 0.9±0.3%, $P < 0.01$, from an occlusion control of 17.1±1.1 mm, and reduced SL stroke shortening by 13.7±3.5%, $P < 0.01$, from an occlusion control of 3.21±0.27 mm, reduced velocity by 15±3%, $P < 0.01$, from an occlusion control of 30.2±2.8 mm/s, and reduced work by 17±4%, $P < 0.01$, (Fig. 4) from an occlusion control of 263±27 mm Hg–mm.

Moderately ischemic zone. Propranolol increased further end-diastolic SL by 0.58±0.18%, $P < 0.01$, from an occlusion control of 18.2±1.0 mm and reduced further SL stroke shortening by 17.2±5.3%, $P < 0.01$, and velocity by 13±4%, $P < 0.01$, from occlusion controls of 1.01±0.12 mm and 12.0±0.9 mm/s, respectively, and reduced work by 7±3%, $P < 0.05$ (Fig. 4), from an occlusion control of 118±15 mm Hg–mm. These figures represent the average values of 18 segments in which function fell, 5 segments in which function improved, and 5 segments in which function did not change. When the average changes in SL stroke shortening, velocity, and work were compared with those in the normal zone in terms of absolute numbers, as opposed to percent change, the decreases in these three parameters were less, $P < 0.01$, than observed for the normal zone.

Severely ischemic zone. Propranolol increased further end-diastolic SL by 0.99±0.21%, $P < 0.01$,

TABLE IV

Regional Blood Flow and ENDO/EPI Ratios: Effects of Coronary Occlusion and Subsequent Propranolol Administration (n = 18) Compared with Untreated Controls (n = 5)

	Preocclusion control	Occlusion	Occlusion and propranolol
Flow, *ml/min/g*			
Normal zone			
Propranolol	1.10±0.03	1.10±0.05	0.93±0.04*‡§
Untreated	1.31±0.06	1.50±0.10	1.48±0.09
Moderately ischemic zone			
Propranolol	0.92±0.03	0.50±0.03*	0.58±0.04*‡§
Untreated	1.27±0.02	0.83±0.05*	0.76±0.07*
Severely ischemic zone			
Propranolol	0.98±0.04	0.19±0.01*	0.26±0.02*‡§
Untreated	1.29±0.03	0.31±0.03*	0.32±0.04*
ENDO/EPI Ratio			
Normal zone			
Propranolol	1.19±0.03	1.22±0.03	1.28±0.03*‡
Untreated	1.20±0.04	1.24±0.04	1.28±0.05
Moderately ischemic zone			
Propranolol	1.22±0.05	0.82±0.06*	0.89±0.07*
Untreated	1.18±0.04	0.88±0.08*	0.93±0.10
Severely ischemic zone			
Propranolol	1.19±0.03	0.63±0.08*	0.64±0.07*
Untreated	1.22±0.06	0.42±0.05*	0.47±0.07*

* Significantly different from preocclusion control, $P < 0.01$.
‡ Significantly different from occlusion value, $P < 0.01$.
§ Response of two groups significantly different, $P < 0.01$.

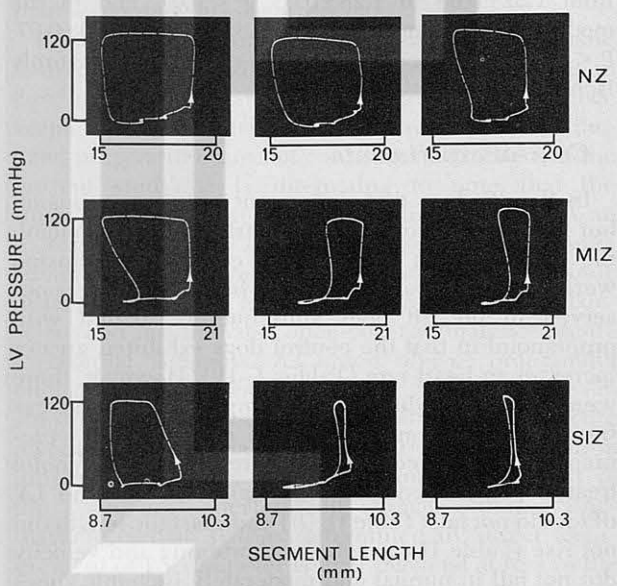


FIGURE 4 Left ventricular (LV) pressure-regional segment length loops, an index of regional myocardial work, are shown for a normal zone (NZ) (top), moderately ischemic zone (MIZ) (middle), and severely ischemic zone (SIZ) (bottom) during control (left panel) after coronary occlusion (middle panel) and after propranolol during coronary occlusion (right panel). Coronary occlusion induced progressively greater decreases in regional work in the three zones. With propranolol work fell further in all three zones. In addition the post-systolic shortening of the ischemic zone segment was reduced by propranolol administration.

from an occlusion control of 17.59±0.70 mm, and reduced paradoxical bulging in the severely ischemic segments, $P < 0.01$. However, function was never improved to the extent that a segment that bulged paradoxically began to shorten during ejection after propranolol administration. Work fell by 40±12%, $P < 0.01$, from an occlusion control of 40±8 mm Hg–mm in segments exhibiting positive work. One of the most prominent effects of propranolol was the reduction in post-systolic shortening in severely ischemic segments shown in the bottom, middle part of Fig. 4.

Intramyocardial Electrogram

Propranolol failed to lower ST elevation in the normal, moderately, or severely ischemic zones from the occlusion levels (Table III).

Regional Myocardial Blood Flow (Table IV)

Propranolol reduced flow in the normal zone by 11±2%, from an occlusion control of 1.10±0.05 ml/min per g and increased flows in the moderately (15 ±4%) and severely ischemic (63±10%) zones from occlusion controls of 0.50±0.03 and 0.19±0.01 ml/min per g, respectively (Fig. 3). These three changes in blood were significant, $P < 0.01$. Propranolol increased the ENDO/EPI ratio in the normal zone from 1.22±0.03 to 1.28±0.03, $P < 0.01$, and in the moderately ischemic zone from 0.82±0.06 to 0.89±0.07, $P < 0.02$, but failed to alter the ratio in the severely ischemic zone.

Control experiments

In the 12 dogs that underwent coronary occlusion but were given normal saline instead of propranolol, the changes from preocclusion control to occlusion were only significantly different from the changes observed in the 18 dogs subsequently treated with propranolol in that the control dogs exhibited greater increases in heart rate (Tables I–IV). However, there were important differences comparing the changes from the occlusion state to the occlusion plus propranolol state. In contrast to the results in propranolol treated dogs, in control dogs, (*a*) heart rate and LV dP/dt did not fall (Table I); (*b*) end-diastolic length did not rise (Table II); (*c*) stroke shortening and velocity did not fall in normal and moderately ischemic zones (Table II); (*d*) the extent of paradoxical bulging did not decrease (Table II); (*e*) regional flow did not fall in the normal zone or rise in the moderately and severely ischemic zone (Table IV).

DISCUSSION

Coronary occlusion resulted in minor effects on overall LV function and function in the normal zone, but induced progressively greater impairment of function in moderately and severely ischemic segments. Propranolol then exerted relatively slight, but statistically significant, effects on regional function in normal, moderately, and severely ischemic zones. In normal segments and in the majority of ischemic segments that still shortened during systole, propranolol reduced the extent and velocity of shortening and segment work performed. In contrast, in those segments that paradoxically lengthened during systole, propranolol decreased the extent of paradoxical motion and post-systolic shortening of those segments (Fig. 4). This latter finding could be interpreted as an improvement in function, since the extent of passive stretching fell after propranolol, but could also merely reflect an interaction between ischemic and nonischemic portions of the heart and may not be directly related to an effect of the drug on the severely ischemic portion of the heart.

Propranolol's depressant action on overall function and function in the normal zone after coronary occlusion was predictable, and is consistent with the prior studies of Mueller et al. (8), and Liang and Hood (7). In contrast its action on ischemic segments could not have been predicted. Studies in open-chest anesthetized animals have shown both improvement (12) and depression of function (10) after beta adrenergic blockers. The study of Theroux et al. (11), conducted in conscious dogs supports that of Lekven (12) in that propranolol pretreatment reduced the impairment of function induced by coronary occlusion on marginally ischemic segments. As mentioned above, in the present study, propranolol administration in doses ranging from 0.5 to 2.0 mg/kg generally depressed function slightly in the presence of sustained coronary occlusion. It is important to point out that the depression induced by propranolol was trivial, e.g., in comparison with the depression induced by simple coronary occlusion (Fig. 2). Moreover, the depression observed in moderately ischemic zones was significantly less than that observed in normal zones.

One of the important differences in the results of this study conducted in conscious dogs and those conducted previously in anesthetized animals with an open chest is the extent to which propranolol reduced heart rate. A striking reduction is frequently observed in anesthetized, open-chest preparations, when propranolol is administered in the face of acute coronary occlusion (1, 2, 5, 6, 10, 13). In contrast heart rate fell by an average of only 8 beats/min after propranolol in the present study. If propranolol were to reduce heart rate considerably more, as occurs in anesthetized animals, a more favorable effect on function of ischemic myocardium may well have been observed. However, in the conscious, unsedated dog with myocardial ischemia, propranolol induces only a slight reduction in heart rate even when the ischemia is relatively large, as occurs with a left circumflex occlusion.

Failure of propranolol to reduce heart rate in the ischemic heart of conscious dogs was also observed by Liang and Hood (7), who suggested that the mechanism of the tachycardia of coronary occlusion was most likely due to withdrawal of vagal restraint. It is interesting to note in the study by Mueller et al. (8), that propranolol reduced heart rate in patients with acute myocardial infarction by an almost identical amount as was observed in the present study in conscious dogs.

While propranolol exerted significant effects on ischemic cardiac function, no significant effect was observed on the ST potential, which has been consistently shown to decrease in experiments conducted in open-chest anesthetized animals (1, 2, 5, 6, 10, 13). It is of interest that Bodenheimer et al. (10), found that the ST electrogram fell substantially in their open-chest anesthetized animals treated with propranolol after coronary occlusion when heart rate was allowed to fall, but returned to the prepropranolol, occlusion level, when the effects of decreased heart rate were eliminated by pacing. These data are consistent with ours, in that heart rate fell by only 8 beats/min in the present experiments, and the ST potential did not fall (Table III).

In the present study, the most striking effects of propranolol were on regional myocardial blood flow. As expected flow fell in normal zones, which probably reflected the reduction in myocardial O_2 demands induced by the reduction in myocardial contractility and work. In contrast, flow rose significantly in moderately and severely ischemic zones despite a reduction in cardiac work and contractility. The mechanism of the redistribution of coronary flow was not examined in the present study. It could have been due to shunting of blood flow from nonischemic to ischemic tissue. Another possible explanation is that forces acting on ischemic myocardium were diminished after propranolol, e.g., post-systolic shortening, thereby allowing more coronary filling. In the severely ischemic zone the redistribution of coronary flow did not favor either the endo or epicardial layers, but occurred transmurally, as reflected by no significant change in the ENDO/EPI ratio. In contrast in the moderately ischemic zone proportionally more flow went to the endocardium, since the ENDO/EPI ratio rose significantly.

Since flow to ischemic tissue can increase spontaneously with time due to opening of collaterals or primary channels from the nonoccluded arteries, it was considered important to conduct a series of control experiments, where saline instead of propranolol was administered. In these experiments flow to ischemic regions did not change significantly over the 10–15-minute period of occlusion studied (Table IV). This is consistent with measurements of regional myocardial function in the present investigation, which also did not change significantly over this 10–15-min period in the animals where ischemia was induced, but saline instead of propranolol, was administered (Table II). These findings are also consistent with prior studies both in conscious and anesthetized dogs. For instance Bishop et al. found that flow to ischemic myocardium did not change between 5 min and 6 h after occlusion in conscious dogs (23), whereas in anesthetized dogs Hirzel et al. (24) and Becker et al. (5) found little change in the central ischemic zone flow from 10 min to 24 h after occlusion in endocardial layers (24) and from 60 to 90 min after occlusion (5), respectively. In contrast, Rivas et al. found that flow to ischemic tissue increased from 45 s to 2 h after occlusion (25). However, the study by Rivas et al. (25) is not inconsistent with the present findings or those of Bishop et al. (23), Hirzel et al. (24), or Becker et al. (5), since all the studies except for that by Rivas et al. (25), made the initial flow determination at a later time after occlusion. Thus, it appears from the results of these studies as well as the control experiments conducted in the present investigation that some flow adjustments normally occur initially (during the first few minutes after occlusion), but the flow to ischemic tissue then remains relatively stable for at least several hours. Therefore, when flow changes significantly during the stable period, it most likely reflects a change induced by the intervention, e.g., propranolol, rather than a spontaneous occurrence.

Prior studies in open-chest anesthetized preparations consistently failed to demonstrate a change in flow to ischemic tissue with propranolol (4–6). Once again, while the results of experiments in anesthetized preparations are not consistent with those of the present study, it is interesting to note that the study by Mueller et al. (8) in patients observed an increase in coronary sinus O_2 tension after propranolol administration. There are two important hemodynamic differences in the response to propranolol in conscious and anesthetized, open-chest animals with acute myocardial ischemia. As noted above, propranolol elicits a much greater decrease in heart rate in anesthetized, open-chest preparations with acute myocardial ischemia (1, 2, 6, 10, 13). In addition propranolol induces greater dilation of the heart of the open-chest anesthetized animal, whereas end-diastolic cardiac size as determined by direct measurement of LV diameter is near maximal at rest in the conscious dog,[3] and thus can only increase slightly with propranolol, as was observed in these experiments.

In summary, propranolol induced a significant redistribution of myocardial blood flow in the ischemic

[3] Boettcher, D. H., S. F. Vatner, G. R. Heyndrickx, and E. Braunwald. Extent of utilization of the Frank-Starling mechanism in control of cardiac performance in the conscious dog. Submitted for publication.

heart with flow falling in normal zones and rising in moderately and severely ischemic zones. This improvement in flow occurred concomitantly with a depression of cardiac function and work and extent of paradoxical bulging or passive stretching in severely ischemic segments. This study suggests two possible salutary actions for propranolol in the treatment of ischemic heart disease where cardiac decompensation is not also a factor. The slight decreases in cardiac rate and contractility should exert an O_2 sparing effect on ischemic myocardium, as long as cardiac failure is not present. This coupled with an increase in blood flow to ischemic tissue could result in protection of ischemic myocardium (1–3) and prove to be beneficial for patients with acute myocardial ischemia.

ACKNOWLEDGMENTS

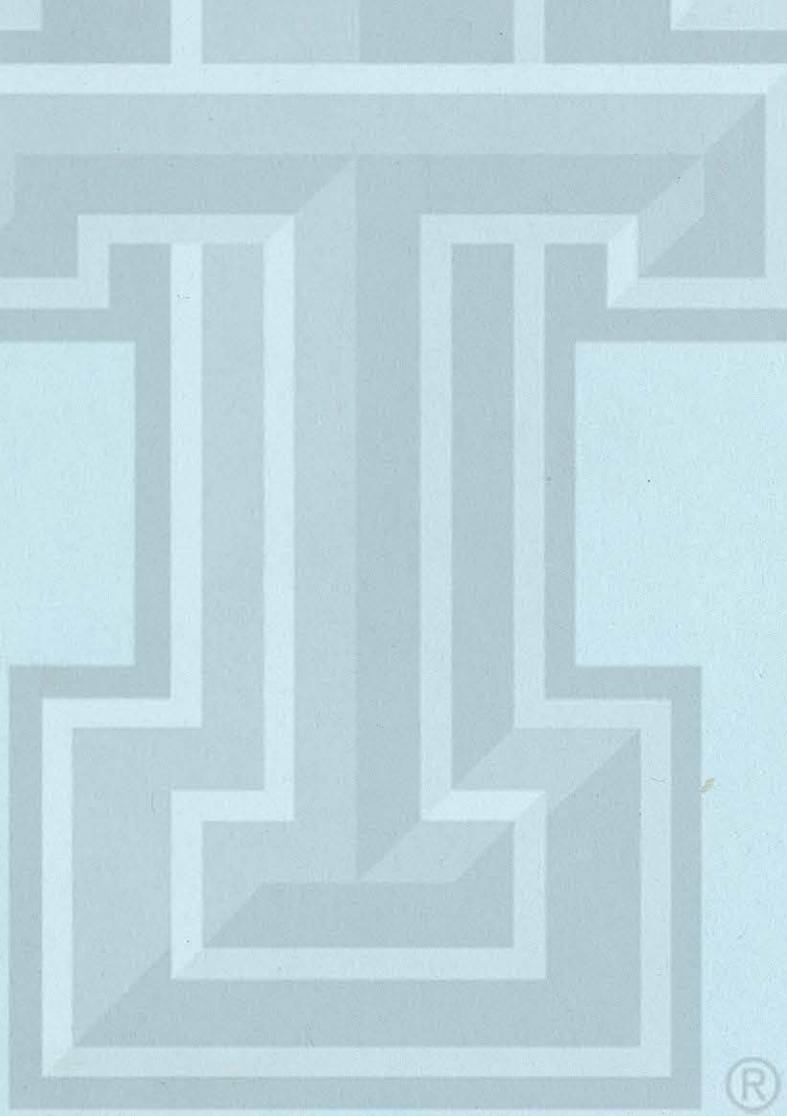
This work was supported, in part, by grants HL 17459, HL 17665, and HL 15416 from the U. S. Public Health Service.

REFERENCES

1. Maroko, P. R., J. K. Kjekshus, B. E. Sobel, T. Watanabe, J. W. Covell, J. Ross, Jr., and E. Braunwald. 1971. Factors influencing infarct size following experimental coronary artery occlusions. *Circulation.* **43:** 67–82.
2. Shatney, C. H., D. J. Maccarter, and R. C. Lillehei. 1976. Effects of allopurinol, propranolol and methylprednisolone on infarct size in experimental myocardial infarction. *Am. J. Cardiol.* **37:** 572–580.
3. Reimer, K. A., M. M. Rasmussen, and R. B. Jennings. 1973. Reduction by propranolol of myocardial necrosis following temporary coronary artery occlusion in dogs. *Circ. Res.* **33:** 353–363.
4. Pitt, B., and P. Craven. 1970. Effect of propranolol on regional myocardial blood flow in acute ischaemia. *Cardiovasc. Res.* **4:** 176–179.
5. Becker, L. C., R. Ferreira, and M. Thomas. 1975. Effect of propranolol and isoprenaline on regional left ventricular blood flow in experimental myocardial ischaemia. *Cardiovasc. Res.* **9:** 178–186.
6. Kloner, R. A., K. A. Reimer, and R. B. Jennings. 1976. Distribution of coronary collateral flow in acute myocardial ischaemic injury: Effect of propranolol. *Cardiovasc. Res.* **10:** 81–90.
7. Liang, C-S., and W. B. Hood, Jr. 1974. The myocardial depressant effect of beta-receptor blocking agents. Comparative study of DL-propranolol, D-propranolol, and practolol in awake dogs with and without acute myocardial infarction. *Circ. Res.* **35:** 272–280.
8. Mueller, H. S., S. M. Ayres, A. Religa, and R. G. Evans. 1974. Propranolol in the treatment of acute myocardial infarction. Effect on myocardial oxygenation and hemodynamics. *Circulation.* **49:** 1078–1087.
9. Kerber, R. E., and D. C. Harrison. 1971. Contraindications and limitations of beta adrenergic blockade in clinical medicine. Circulatory effects and clinical uses of beta-adrenergic blocking drugs. *Excerpta Med. Int. Congr. Ser.* 131–138.
10. Bodenheimer, M. M., V. S. Banka, and R. H. Helfant. 1976. Propranolol in experimental myocardial ischemia: Dissociation of effects on contraction and epicardial ST segments. *Am. Heart J.* **92:** 481–486.
11. Theroux, P., J. Ross, Jr., D. Franklin, W. S. Kemper, and S. Sasayama. 1976. Regional myocardial function in the conscious dog during acute coronary occlusion and responses to morphine, propranolol, nitroglycerin, and lidocaine. *Circulation.* **53:** 302–314.
12. Lekven, J. 1975. Effect of practolol on left ventricular dimensions during coronary occlusion. *Am. J. Cardiol.* **36:** 179–184.
13. Wendt, R. L., R. C. Canavan, and R. J. Michalak. 1974. Effects of various agents on regional ischemic myocardial injury: Electrocardiographic analysis. *Am. Heart J.* **87:** 468–482.
14. Manders, W. T., and S. F. Vatner. 1976. Effects of sodium pentobarbital anesthesia on left ventricular function and distribution of cardiac output in dogs, with particular reference to the mechanism for tachycardia. *Circ. Res.* **39:** 512–517.
15. Vatner, S. F., and N. T. Smith. 1974. Effects of halothane on left ventricular function and distribution of regional blood flow in dogs and primates. *Circ. Res.* **34:** 155–167.
16. Franklin, D. E., N. W. Watson, K. E. Pierson, and R. L. Van Citters. 1966. Technique for radio telemetry of blood-flow velocity from unrestrained animals. *Am. J. Med. Electron.* **5:** 24–28.
17. Vatner, S. F., D. Franklin, and R. L. VanCitters. 1970. Simultaneous comparison and calibration of the Doppler and elegromagnetic flowmeters. *J. Appl. Physiol.* **29:** 907–910.
18. Patrick, T. A., S. F. Vatner, W. S. Kemper, and D. Franklin. 1974. Telemetry of left ventricular diameter and pressure measurements in unrestrained animals. *J. Appl. Physiol.* **37:** 276–281.
19. Vatner, S. F., R. W. Millard, T. A. Patrick, and G. R. Heyndrickx. 1976. Effects of isoproterenol on regional myocardial function, electrogram, and blood flow in conscious dogs with myocardial ischemia. *J. Clin. Invest.* **57:** 1261–1271.
20. Domenech, R. J., J. I. E. Hoffman, M. I. M. Nobel, K. B. Saunders, J. R. Henson, and S. Subijanto. 1969. Total and regional coronary blood flow measured by radioactive microspheres in conscious and anesthetized dogs. *Circ. Res.* **25:** 581–596.
21. Millard, R. W., H. Baig, and S. F. Vatner. 1977. Cardiovascular effects of radioactive microsphere suspensions and Tween 80 solutions. *Am. J. Physiol.* **1:** H331–334.
22. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 6th edition. 91–98.
23. Bishop, S. P., F. C. White, and C. M. Bloor. 1976. Regional myocardial blood flow during acute myocardial infarction in the conscious dog. *Circ. Res.* **38:** 429–438.
24. Hirzel, H. O., G. R. Nelson, E. H. Sonnenblick, and E. S. Kirk. 1976. Redistribution of collateral blood flow from necrotic to surviving myocardium following coronary occlusion in the dog. *Circ. Res.* **39:** 214–222.
25. Rivas, F., F. R. Cobb, R. J. Bache, and J. C. Greenfield, Jr. 1976. Relationship between blood flow to ischemic regions and extent of myocardial infarction. Serial measurement of blood flow to ischemic regions in dogs. *Circ. Res.* **38:** 439–447.

Cardiovascular effects of radioactive microsphere suspensions and Tween 80 solutions

RONALD W. MILLARD, HANK BAIG, AND STEPHEN F. VATNER
Department of Medicine, Harvard Medical School and Peter Bent Brigham Hospital, and Department of Cardiology, Children's Hospital Medical Center, Boston 02115; and New England Regional Primate Research Center, Southborough, Massachusetts 01772



Cardiovascular effects of radioactive microsphere suspensions and Tween 80 solutions

RONALD W. MILLARD, HANK BAIG, AND STEPHEN F. VATNER
Department of Medicine, Harvard Medical School and Peter Bent Brigham Hospital, and Department of Cardiology, Children's Hospital Medical Center, Boston 02115; and New England Regional Primate Research Center, Southborough, Massachusetts 01772

MILLARD, RONALD W., HANK BAIG, AND STEPHEN F. VATNER. *Cardiovascular effects of radioactive microsphere suspensions and Tween 80 solutions*. Am. J. Physiol. 232(3): H331–H334, 1977 or Am. J. Physiol.: Heart Circ. Physiol. 1(3): H331–H334, 1977. —The cardiovascular effects of two concentrations of Tween 80 (polyoxyethylene sorbitan mono-oleate), a surface-active agent commonly used to prevent aggregation of radionuclide-labeled microspheres, were examined in conscious dogs. Two types of adverse reactions were noted. The first (*Type A*) consisted of reductions in cardiac dimensions as well as hypotension and tachycardia. The second (*Type B*) was less severe and involved only a decrease in cardiac dimensions with no change in left ventricular systolic pressure or heart rate. A 10% dextran solution with .05 ± .02% Tween 80 injected into the left atrium caused systemic and/or cardiac alterations in all four dogs studied. Administration of a lower concentration of Tween 80 (0.01 ± 0.005%), which was the minimum concentration necessary to prevent aggregation of microspheres, induced adverse reactions in 6 of 41 dogs studied. Subsequent administration of this concentration of Tween 80 on the same day rarely induced adverse reactions. Thus, care must be exercised in application of microsphere techniques to organ blood flow measurements when Tween 80 is used to prevent microsphere aggregation, since this surface-active agent causes profound alterations in cardiac dynamics in concentrations normally employed in experiments involving microsphere techniques.

cardiac volume; radionuclide; myocardial blood flow; cardiac dynamics

---

RADIONUCLIDE-LABELED microspheres are widely used to assess the distribution of regional blood flows (7, 13) and distribution of blood flow across the myocardial wall (3–6, 9). Many potential sources of error for this technique have been analyzed in detail. For instance, Buckberg et al. (3) have defined requirements for speed of arterial reference blood withdrawal and number and distribution of microspheres in tissue for statistically valid flow determination. Another possible but important source of error is recognized, i.e., formation of large aggregates of microspheres, which would result in both nonuniform distribution of the spheres and might also cause hypotension if major channels to the heart and brain were occluded. For the prevention of aggregation, the microspheres are generally kept in suspension by addition of the surface-active, polyoxyethylene sorbitan monooleate (Tween 80; Fisher Scientific Co., Pittsburgh). Since the application of microspheres to the study of the circulation assumes that the microsphere suspension does not alter cardiovascular dynamics, it is surprising that despite previously demonstrated hemodynamic actions of Tween (4, 10), little attention has been focused on the effects of this agent, per se, when microsphere techniques are employed.

The results from preliminary experiments in our laboratory in conscious dogs indicated that significant hemodynamic changes occurred subsequent to left atrial injection of microspheres, which were suspended in 0.05% Tween 80–10% dextran and whose dispersion was verified by microscopic examination. To determine the cause of these adverse reactions we examined responses of conscious dogs to solutions of 10% dextran and Tween 80 with and without microspheres. Since microspheres are frequently used to assess intramyocardial distribution of coronary blood flow, particular attention was paid to the effects of left ventricular pressures and dimensions, i.e., important determinants of myocardial oxygen consumption and consequently coronary blood flow (2).

METHODS

Mongrel dogs of either sex, 20–25 kg, were anesthetized with sodium pentobarbital, 30 mg/kg. The instrumentation was implanted through a left thoracotomy in the fifth intercostal space. Catheters were inserted into the left atrium for injection of microsphere suspensions and Tween solutions. Left ventricular pressure was measured with an implanted miniature solid-state gauge (Konigsberg P 22; Konigsberg Instruments Inc., Pasadena, Calif.). A segment length of the left ventricle was measured with a pair of miniature (1 mm) ultrasonic crystals implanted intramyocardially 1–2 cm apart to assess regional myocardial segment length (40 dogs)

or a pair of crystals implanted across the ventricular wall to measure wall thickness (three dogs) (8, 12).

All animals were allowed to recover from operation for a period of 1–4 wk before study. When the conscious dogs were resting quietly, recordings of left ventricular pressure, segment length, and heart rate were made. Radioactive microspheres (3M Co., St. Paul, Minn.) of either 15 ± 5 μm or 9 ± 1 μm in diameter were suspended in solutions of either .05 ± .02% or .01 ± .005% Tween 80 in 10% dextran. Aliquots (0.7–2 ml) of these suspensions were injected in the left atrium, followed by 2–5 ml of physiologic saline. To determine if the vehicle, rather than the microspheres, was responsible for the alterations in cardiovascular hemodynamics, the supernatant derived from the suspensions by centrifugation was injected into the left atrium in different dogs. To determine if dextran alone was responsible, dextran was injected into the left atrium.

In a final series of experiments microsphere suspensions that were in .05% Tween 80 for several weeks were centrifuged. The supernatant was drawn off and replaced with saline. This procedure was repeated and the supernatant derived from the second centrifugation was injected into the left atrium of conscious dogs.

RESULTS

Two types of adverse reactions were observed. The first (*Type A*) was more severe and involved systemic as well as cardiac changes (Fig. 1). Left ventricular (LV) systolic pressure fell from 116 ± 4 to 85 ± 12 mmHg, while heart rate rose from 101 ± 7 to 146 ± 7 beats/min. LV end-diastolic pressure fell from 7 ± 1 to 3 ± 1 mmHg, while LV end-diastolic dimension fell by 17 ± 6% from a control of 12.05 ± 1.38 mm and wall thickness rose by 13%. In the seven animals in which this type of reaction was observed, these changes occurred from 0.5 to 2.0 min after the injection, were all significant, $P < 0.01$, and returned to control between 2 and 25 min. More prolonged reactions were observed in animals not included in this study, where more than 2 ml of .05% Tween was injected in the left atrium.

The second type of reaction (*Type B*) involved only decreases in cardiac dimensions and LV end-diastolic pressure while LV systolic pressure and heart rate did not change significantly. In the six animals in which this reaction was observed LV end-diastolic dimension fell by 5.3 ± 1.2% from a control of 15.70 ± 0.99 mm and wall thickness rose by 5% while LV end-diastolic pressure fell from 10 ± 2 to 6 ± 1 mmHg. Peak dP/d*t* did not change significantly, which, in the face of reduced preload, may reflect a slight increase in the inotropic state. Figure 2 shows a typical *Type B* response. These changes occurred from 0.5 to 2.0 min and returned to control within 5 min.

The number of dogs exhibiting each type of reaction is shown in Table 1. Microsphere-free supernatant from centrifuged suspensions produced similar hemodynamic reactions as did the suspensions themselves, indicating that the Tween 80 solutions rather than the microspheres, per se, were responsible for the adverse reactions. This response was also obtained with Tween 80 solutions never in contact with microspheres. However, prolonged contact between Tween solutions and microspheres seemed to increase the probability of adverse reactions, since .05% Tween 80-microsphere suspen-

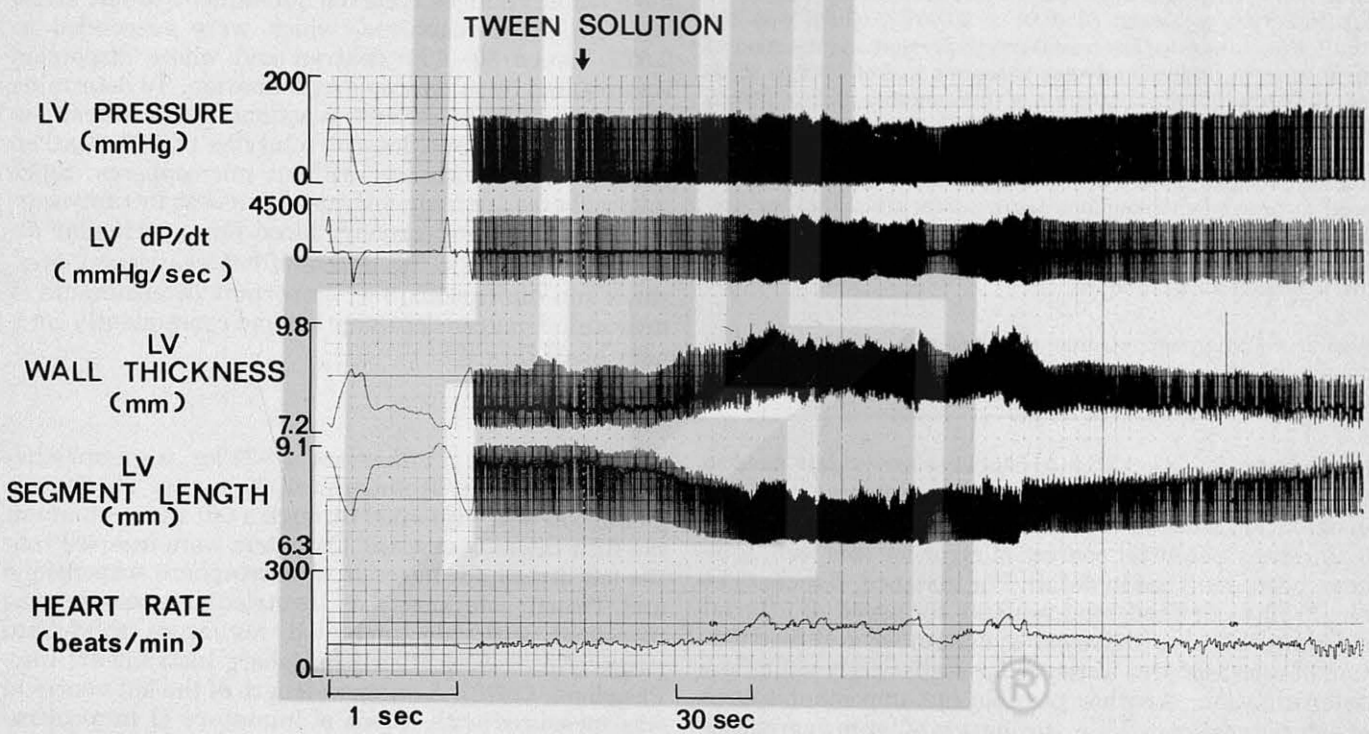


FIG. 1. Typical *Type A* response to injection of 0.05% concentration of Tween 80 in 10% dextran solution through a left atrial catheter is shown to induce hypotension, tachycardia, and a substantial reduction in left ventricular end-diastolic segment length together with a reciprocal increase in wall thickness.

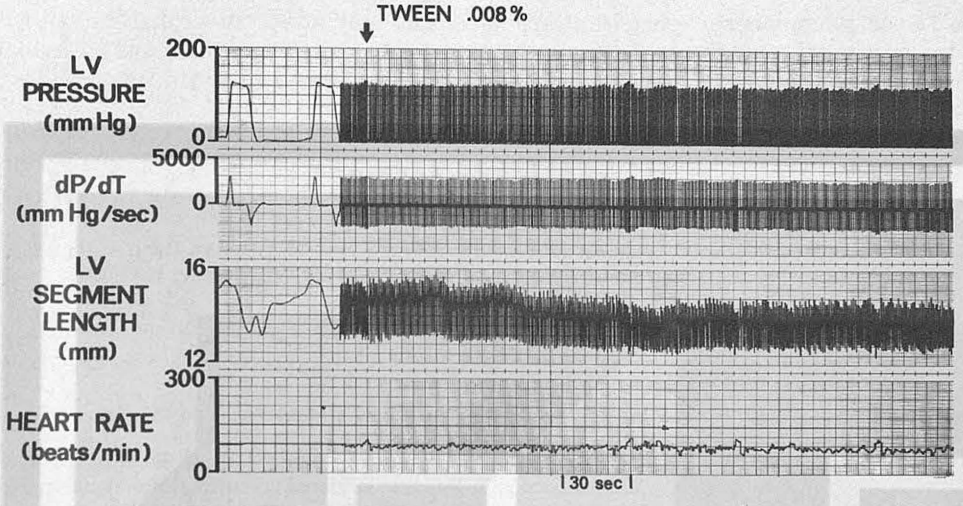


FIG. 2. *Type B* reaction showing a decrease in regional myocardial dimensions without associated changes in heart rate or left ventricular systolic pressure, when 0.01% concentration of Tween 80—10% dextran solution was injected into left atrium in a conscious dog.

TABLE 1. *Adverse reactions to Tween 80*

	Total Number of Animals Studied	Number of Reactions Observed	
		Type A	*Type B*
1) 0.05% Tween solution	4	2	2
2) Aged microsphere-Tween suspension*	8	2	1
3) 0.01% Tween solution with microspheres	25	3	2
4) 0.01% Tween solution	16	0	1

* Microspheres were suspended in a .05% Tween and held for 2–4 wk. Prior to injection, the microsphere suspension was centrifuged twice and replaced with saline (no Tween) each time. After the second saline replacement, the suspension was centrifuged and the supernatant was injected.

sions, which had been in contact for several weeks, but prior to injection had been centrifuged and washed twice with saline, still frequently produced adverse reactions (Table 1). Dextran alone did not produce detectable hemodynamic changes. When dry microspheres were freshly suspended in .01% Tween 80 solution, adverse reactions were rarely noted.

The occurrence of adverse reactions subsequent to .05% Tween 80 solution injections could not be repeated in the same animal on subsequent injections in the same day, despite return of cardiac and hemodynamic parameters to preinjection control values. However, 24–48 h after the initial reaction a second adverse reaction could be evoked with injection of Tween 80 solution.

DISCUSSION

Tween has been reported to have extremely low toxicity; subcutaneous injections in rats have failed to significantly increase mortality until a dose of 0.25 ml of 12% Tween 80 solution was injected each week for 27 wk (11). However, a related polyoxylated fatty acid derivative (Tween 20) has been reported to release endogenous stores of histamine, increase capillary permeability, and result in an anaphylacticlike syndrome (4, 10). The similarity between these findings and those reported in this paper are noteworthy, especially considering the *Type A* reaction, which involved hypotension. Hypotensive reactions subsequent to microsphere injections have been noted by others as well (5), but have not been attributed to the surfactant. Since Tween 80 solutions, both with and without microspheres, produced similar hemodynamic changes in the present study, it was concluded that the surfactant, rather than the microspheres, were responsible for the adverse reactions.

In addition to the reaction involving systemic hemodynamic changes (*Type A*), we observed milder adverse reactions involving a reduction in cardiac dimensions without detectable systemic effects (*Type B*). The effect of the surfactant would have been overlooked in these cases if cardiac dimensions were not measured (Fig. 2). Moreover, the effect would have been more difficult to observe in the open-chest anesthetized dog, where cardiac size is diminished (14). The observation in conscious dogs that cardiac size fell while left ventricular systolic pressure and heart rate did not change implies that wall tension fell, which would lower myocardial oxygen requirements and consequently alter myocardial blood flow. This latter aspect gains importance in view of the fact that suspensions of radioactive microspheres and Tween 80 are widely used to determine the distribution of intramyocardial blood flow (1,5,6,9).

The onset of the observed cardiac size changes or systemic hypotension occurred from 30 s to 2 min after the beginning of microsphere injections. Potential errors in regional myocardial flow determinations should be anticipated in cases in which the adverse effect had an early onset (30 s), but should be minimal in cases in which cardiovascular effects occurred later (2 min) since the microspheres should have been distributed prior to the change in hemodynamics. However, in both instances, subsequent flow determinations would be seriously compromised if an injection were undertaken during recovery from the adverse response. Subsequent injections of microspheres should be carried out only after cardiac size, arterial pressure, heart rate, and indices of contractility have returned to control, which in these animals occurred within 0.5 h, but in other animals occurred much later where more than 2 ml of .05% Tween was injected.

The observed adverse reactions to Tween-microsphere suspensions were determined to be due both to the amount of Tween injected and to the length of time that the Tween solution remained in contact with the microspheres. Accordingly, to avoid these adverse reactions, we use a minimal level of Tween 80 (.01%) to suspend the microspheres. The solution is then agitated for 30 min by ultrasonic probe and placed in an ultrasonic bath until the moment of injection. Dispersion of the microspheres is verified by microscopic examination. Since the length of time during which microspheres and supernatant are in contact may affect the probability that an adverse reaction will occur, fresh microsphere suspensions are prepared every 3 days to minimize undesirable hemodynamic responses.

This study was supported in part by Public Health Service Grants HL-17459, HL-15416, and HL-17665.

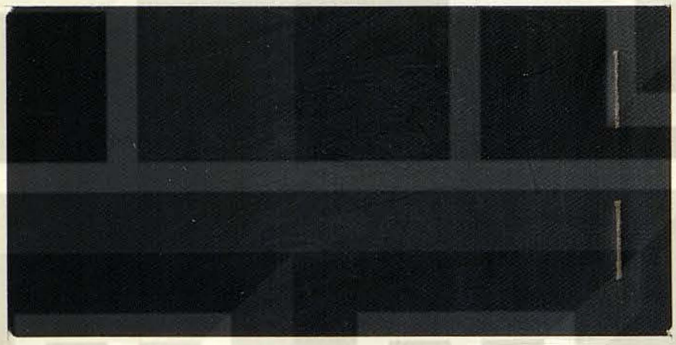
S. F. Vatner is an Established Investigator, American Heart Association.

Address requests for reprints to R. W. Millard, Dept. of Physiology and Biophysics, Brown University, Providence, R.I. 02912.

Received for publication 29 July 1976.

REFERENCES

1. BECKER, L. C., R. FERREIRA, AND M. THOMAS. Effect of propranolol and isoprenaline on regional left ventricular blood flow in experimental myocardial ischemia. *Cardiovascular Res.* 9: 178–186, 1975.
2. BRAUNWALD, E., J. ROSS, JR., AND E. H. SONNENBLICK. *Mechanisms of Contraction of the Normal and Failing Heart.* Boston: Little, Brown, 1967.
3. BUCKBERG, G. D., J. C. LUCK, D. B. PAYNE, J. I. E. HOFFMAN, J. P. ARCHIE, AND D. E. PYLER. Some sources of error in measuring regional blood flow with radioactive microspheres. *J. Appl. Physiol.* 31: 598–604, 1971.
4. BURNELL, R. H., AND G. M. MAXWELL. General and coronary hemodynamic effects of Tween 20. *Australian J. Exptl. Biol. Med. Sci.* 52: 151–156, 1974.
5. COBB, F. R., R. J. BACHE, AND J. C. GREENFIELD, JR. Regional myocardial blood flow in awake dogs. *J. Clin. Invest.* 53: 1618–1625, 1974.
6. DOMENECH, R. J., J. I. E. HOFFMAN, M. I. M. NOBLE, K. B. SAUNDERS, J. R. HENSON, AND S. SUBIJANTO. Total and regional blood flow measured by radioactive microspheres in conscious and anesthetized dogs. *Circulation Res.* 25: 581–594, 1969.
7. FORSYTH, R. P., B. I. HOFFBRAND, AND K. L. MELMON. Redistribution of cardiac output during hemorrhage in the unanesthetized monkey. *Circulation Res.* 27: 311–320, 1970.
8. HEYNDRICKX, G. R., R. W. MILLARD, R. J. MCRITCHIE, P. R. MAROKO AND S. F. VATNER. Regional myocardial functional and electrophysiological alterations following brief coronary artery occlusion in conscious dogs. *J. Clin. Invest.* 56: 978–985, 1975.
9. KJEKSHUS, J. K. Mechanism for flow distribution in normal and ischemic myocardium during increased ventricular preload in the dog. *Circulation Res.* 33: 489–499, 1973.
10. MARKS, L. S., AND S. N. KOLMEN. Tween 20 shock in dogs and related fibrinogen changes. *Am. J. Physiol.* 220: 218–221, 1971.
11. MATSUYAMA, M., AND K. SUZUMORI. Effects of repeated subcutaneous injection of Tween 80 in rats. *Nagoya Med. J.* 12: 1–6, 1966.
12. PATRICK, T. A., S. F. VATNER, W. S. KEMPER, AND D. FRANKLIN. Telemetry of left ventricular diameter and pressure measurements from unrestrained animals. *J. Appl. Physiol.* 37: 276–281, 1974.
13. RUDOLPH, A. M., AND M. A. HEYMANN. The circulation of the fetus in utero. *Circulation Res.* 21: 163–184, 1967.
14. RUSHMER, R. F. Shrinkage of the heart in anesthetized, thoracotomized dogs. *Circulation Res.* 2: 22–27, 1954.



Lockheed

MISSILES & SPACE COMPANY

A GROUP DIVISION OF LOCKHEED AIRCRAFT CORPORATION

SUNNYVALE, CALIFORNIA

AN EVALUATION OF CARDIOVASCULAR DECONDITIONING COUNTERMEASURES

Introduction

The anticipated cardiovascular and muscular deconditioning which will be experienced by astronauts during prolonged space flight is of increasing importance. Concern exists over the possibility of increased susceptibility to syncope, especially during critical re-entry and early post landing periods. Whether this orthostatic intolerance is secondary to low-g effects ("prolonged weightlessness") or merely to enforced motor inactivity has not been resolved. This technical brief describes the LMSC's recent interest in the use of lower body negative pressure and exercise as specific countermeasures to deconditioning effects.

Background

Over the past few years there has been considerable research accomplished in an effort to find effective countermeasures to weightlessness and the confinement of space travel. The principle forms of deconditioning to be considered here include hemoconcentration with a reduction of blood volume, orthostatic intolerance, and a reduction of work capacity as evidenced by a loss of work performance.

Miller (1965) studied the use of moderate exercise (bicycle-like device) as a deconditioning countermeasure during four weeks of bed rest. He found that the exercising group did as poorly as the control group as evidenced by blood volume losses (average of 1212 ml), orthostatic intolerance, and reduced work capacity. He concluded that his exercise program did not sufficiently stress the circulatory system.

Vogt and Vallbona (1965) did an extensive evaluation of absolute bed rest contrasted with bed rest plus isometric exercise. The exercise consisted of a thrust force equal to 300 lbs and was performed six times a day. A moderate protection of orthostasis was demonstrated by a less steep change in the slopes of heart rate and blood pressure in the exercise group. There was a decrease in blood volume during the bed rest/exercise program as compared to the absolute bed rest sequence. Ability to maintain a baseline work load on the bicycle ergometer was improved in the bed rest/exercise group.

Cooper (1966) has shown that pre-training with vigorous supine exercise (bicycle ergometry), and exercise during bed rest at a level of 750 to 1,000 KCal/day, exerts a moderate protective effect on the cardiovascular system. He also observed significant maintenance in work capacity and less tendency towards hemoconcentration..

Venous occlusive cuffs have been used in two principle studies.

Vogt (1965) demonstrated protection of plasma volume and orthostatic intolerance during a six hour water immersion study when cuffs inflated to 60 mm Hg were applied to the upper thigh in a one minute on - one minute off sequence. In this study he used four subjects who served as their own controls. As controls, three of the four had syncopal reactions on the tilt table. When using the cuffs, the subjects responded about as well as their pre-test baseline.

Stevens (1966) deconditioned subjects for 28 days and then applied cuffs inflated to 40 mm Hg to the upper thigh continuously for 16 hours per day for four days. Plasma volume was replenished from 290 ml to 100 ml below baseline but orthostatic intolerance persisted.

Stevens (1965) discussed the mechanism of using lower body negative pressure (LBNP). He established what levels of negative pressure could be tolerated by subjects. At -80 mm Hg all subjects developed symptoms of impending syncope, at -60 mm Hg 30% of the subjects lasted 20 minutes, at -40 mm Hg 42% survived, and at -25 mm Hg all subjects tolerated the procedure. This work was done preliminary to using LBNP as a deconditioning countermeasure.

Lamb (1965) investigated only the effect of LBNP on experimentally produced dehydration. He found that two days of 12 hour per day application of LBNP replenished the plasma volume losses and returned the hematocrit levels to normal. In this initial study he did not investigate changes in orthostatic intolerance.

Stevens (1966) conducted an extensive evaluation of LBNP with a total of 40 subjects. Positive results of maintenance of plasma volume levels, work performance (treadmill tolerance), and orthostatic reflexes

were found in each of the following evaluations.

1. The use of LBNP daily during 28 days of bed rest. The LBNP was used 10 hours per day, cycled between 50 mm Hg for four minutes and 25 mm of Hg for two minutes.
2. The use of LBNP, after 11 days of absolute bed rest, for three days at -30 mm Hg for 10 hours per day.
3. The use of LBNP after 28 days of absolute bed rest at 10,000 and 12,000 feet simulated altitude. The LBNP was used for two days at -30 mm Hg for 10 hours per day.

As these studies suggest, there are several unexplored areas in relationship to using countermeasure devices under actual space flight conditions. As yet the minimum quantity of negative pressure and length of exposure time needed for the prevention of cardiovascular deconditioning is far from adequately determined. It is anticipated that one person, unassisted, could operate the chamber using appropriately designed safety switches; however, this needs further investigation. The use of a bicycle ergometer does help in preventing muscular deconditioning. Brief periods of vigorous exercise has been shown to prevent drastic degradation in work performance during deconditioning experiments. Optimum scheduling and length of the exercise periods still needs delineation.

The LMSC Biotechnology organization has developed prototype flight models of both a bicycle ergometer and a lower body negative pressure (LBNP) chamber (Figure 1) under Contract #AF41(609)-2800; "Development of Space Flight Devices to Diminish or Prevent the Deconditioning Effects of Weightlessness". The equipment is currently undergoing investigation at the USAF School of Aviation Medicine under the direction of Major K. Cooper (MC) USAF. An operational difficulty with the prototype LBNP

chamber has been the rubber gasket subject seal (Figure 2) separating at high negative pressure (-60 to -80 mm Hg). LMSC has developed an improved rubber gasket incorporating a layer of stretchable fibers which has performed satisfactorily even at high negative pressure levels. In addition, the integrated use of the LBNP chamber and bicycle ergometer has been proposed as a deconditioning countermeasure.

LMSC Recent Efforts

A recent company funded effort has been to investigate the use of the integrated LBNP chamber/bicycle ergometer as a countermeasure to prevent muscular and cardiovascular deconditioning. Experiments were done to evaluate any synergistic effect between LBNP and exercise which might decrease the time needed to prevent deconditioning.

1. Abbreviated Protocol

   Rapid 36-48 hour deconditioning of four trained volunteer subjects was done using a combined water immersion/bed rest technique (six hour periods of salt water immersion alternating with 18 hours of bed rest). See Figure 3. Prior to the actual test there was a six week training period during which baseline data was obtained for the blood pressure and heart rate responses to the tilt table test and exercise on the ergometer. Half of the subjects (experimental group) spent two hour periods, twice a day, in the combined LBNP/Ergometer device. During these two hour periods the negative pressure was varied between -20 mm Hg and -30 mm Hg and there were six evenly spaced exercise periods of five minutes duration each. Pre- and post-experiment testing included tilt table endurance (80° head up tilt for 21 minutes), work performance using an upright ergometer, resting O_2 consumption, vital capacity, plasma volume (RISA), hemogram, and a controlled Valsalva maneuver (40 mm Hg flack test). Medical monitoring of the EKG, heart rate, core temperature (external auditory canal), and blood pressure was conducted using standard bioinstrumentation methods.

2. Results

   A full report of the experiments is being prepared. Briefly, the results are as follows.

A. Within 48 hours, using our combined water immersion/bed rest technique, previously well conditioned subjects showed considerable cardiovascular and muscle deconditioning. This was demonstrated primarily by a decreased plasma volume, decreased exercise tolerance (ergometer), and decreased tilt table tolerance.

B. Countermeasures to deconditioning carried out during the test period by using a combined LBNP/Bicycle Ergometer prevented some muscle deconditioning. The experimental subjects (exercise) were able to perform equally well on the upright ergometer after the test as before. The control subjects (no exercise) were not able to perform at pre-test standards. See Figure 4.

C. However, the combined exercise/negative pressure exposure during the test period did not appear to protect the experimental subjects from cardiovascular deconditioning. See Figure 5. Why this combination was not effective is not clear. The small sample size made the experiment subject to the known variability in using biological samples. It is possible that concomitant use of the bicycle ergometer and LBNP chamber may be acting in an antagonistic manner rather than a synergistic manner. The maximum pressure used (-30 mm Hg) may not have been severe enough, or its period of application long enough to have an effect. The optimum degree of LBNP application to prevent or reduce the cardiovascular deconditioning effects of weightlessness must be established. In addition, this regime must be compatible with realistic astronaut work-rest cycles.

Technical Approach

A 10 day combined bed rest/water immersion deconditioning experiment using countermeasures is proposed. The objects of the experiment are as follows.

1. Investigate the use of the lower body negative pressure (LBNP) chamber as a brief diagnostic test to quantitatively detect in-flight cardiovascular deconditioning.
2. Investigate the benefit of intensive end-of-flight reconditioning using the LBNP chamber during the sleep phase of a normal work/rest cycle.
3. Demonstrate the benefit of brief, daily supine exercise with a bicycle ergometer during flight in maintaining work performance by preventing muscular deconditioning.

The reasons for reaching these objectives are as follows: As yet, no quantitative daily detection of the amount of cardiovascular deconditioning an astronaut is experiencing has been possible. Using the LBNP chamber as a diagnostic test, a method of repeatedly testing vascular reflexes normally operative under a 1-g environment would be possible. The supine LBNP chamber would then be substituting as a simplified in-flight tilt table test. The parameters for quantitating the amount of deconditioning that is occuring would be the change in heart rate and blood pressure measurements as compared to on ground baseline measurements. Once quantification of deconditioning has been established, the same LBNP chamber could then be used as treatment to counteract the deconditioning. When it is shown that cyclic LBNP can safely be applied to an astronaut during six hours of sleep and be effective against cardiovascular deconditioning it's use in a spacecraft will be simplified. And similarly, an experiment demonstrating the benefit of brief periods of vigorous supine exercise in

preventing significant muscle deconditioning would be accomplished.

An experiment designed to accomplish these objects is as follows.

1. Four volunteer subjects who have passed the Class II Air Force physical will be chosen.
2. A pre-training evaluation will be as follows.
   A. Each subject will sleep in the LBNP chamber for six hours. During this period the negative pressure will be cycled hourly from -40 mm Hg to 0 mm Hg and back to -40 mm Hg again. This will demonstrate the safety in having an untrained subject sleep in the LBNP chamber.
   B. A hemogram and urinalysis will be obtained.
3. A six week training period will be as follows for each subject.
   A. Exercise daily for seven minutes on the supine bicycle ergometer (200 watts).
   B. Twice a week spend 30 minutes in the LBNP chamber with a setting of -40 mm Hg. ("Diagnostic LBNP test")
   C. Twice a week spend 30 minutes on the tilt table at 90° head up tilt.
   D. Obtain working oxygen consumptions (ergometer test).
4. The 10 day experiment period will be as follows.
   A. Pre-experiment test data will consist of:
      (1) Baseline values of blood pressure and heart rate obtained during the training period for the 30 minute LBNP chamber test, tilt table test, and supine bicycle ergometer exercise.
      (2) Plasma volume, hemogram and urinalysis.
      (3) Working oxygen consumption on the ergometer.
   B. The subjects will spend 10 days at bed rest with daily four hour periods immersed in salt water to hasten and enhance deconditioning.

C. Daily the subjects will:

(1) Twice exercise on the supine bicycle ergometer for seven minutes at 200 watts. Working oxygen consumption will be obtained concurrently.

(2) Have a diagnostic 30 minute LBNP chamber test at -40 mm Hg.

The blood pressure and heart rate values for the above procedure, plus the oxygen consumption, when compared to the pre-experiment baseline values would allow a quantitative assessment of the amount of deconditioning that is occurring.

D. On the seventh day (three days prior to the end of the test), treatment with the LBNP chamber will commence. At night, each subject will spend six hours sleeping in a LBNP chamber with cyclic negative pressure applied. During the day, diagnostic assessment of the amount of reconditioning will continue to be done using the 30 minute LBNP test.

E. Post-experiment test data will consist of tilt table test results, working oxygen consumption (ergometer), and plasma volume.

The results will show the value in the use of the bicycle ergometer and the lower body negative pressure chamber as effective countermeasures to the deconditioning effects of prolonged weightlessness.

Scope of Effort

The project would extend over six months, require 4,000 man hours and $8,000 in materials (includes fabrication of four LBNP chambers).

Bibliography

1. Miller, P. B., Johnson, R. L., and Lamb, L. E., "Effect of Moderate Physical Exercise During Four Weeks of Bed Rest on the Circulatory Function in Man", Aerospace Med 36 1077-1082 (1965)
2. Vallbona, C., Vogt, F. B., Cardus, D., Spencer, W. A., and Walters, M., "The Effect of Bedrest on Various Parameters of Physiological Function", Vol. VIII, VII and IX NASA Report CR-171-184 (Mar. 1965)
3. Cooper, K. H., et al, "A Study Designed to Determine the Effectiveness of Vigorous Supine Exercise in Preventing Bed Rest Deconditioning - Tilt Table Tolerance, Work Capacity and Pulmonary Indices", USAF, SAM, Brooks AFB, Texas, Presented at the Aerospace Medical Association Meeting, Las Vegas, Nevada, May 1966.
4. Vogt, F. B., "Effect of External Cuffs in Tilt Table Tolerance After Water Immersion", Aerospace Med 36 442-447 (1965).
5. Stevens, P. M., Lynch, T. N., Johnson, R. L., and Lamb, L. E., "Effects of 9-Alphafluorohydrocortisone and Venous Occlusive Cuffs and Orthostatic Deconditioning of Prolonged Bed Rest", Aerospace Med 37 1049-1056 (1966)
6. Stevens, P. M., and Lamb, L. E., "Effects of Lower Body Negative Pressure on the Cariovascular System", American Journal of Cardiology 16 (1965)
7. Lamb, L. E., and Stevens, P. M., "Influence of LBNP on Levels of Hydration During Bed Rest", Aerospace Med 36 (1965)
8. Stevens, P. M., Miller, P. B., and Lamb, L. E., "Potential Uses of Lower Body Negative Pressure as an Anti-Deconditioning Measure During Weightlessness", USAF SAM, Brooks AFB, Texas, Presented at the Aerospace Medical Association Meeting, Las Vegas, Nevada, May 1966

Figure 1

Figure 2

DECONDITIONING
BED REST & WATER IMMERSION

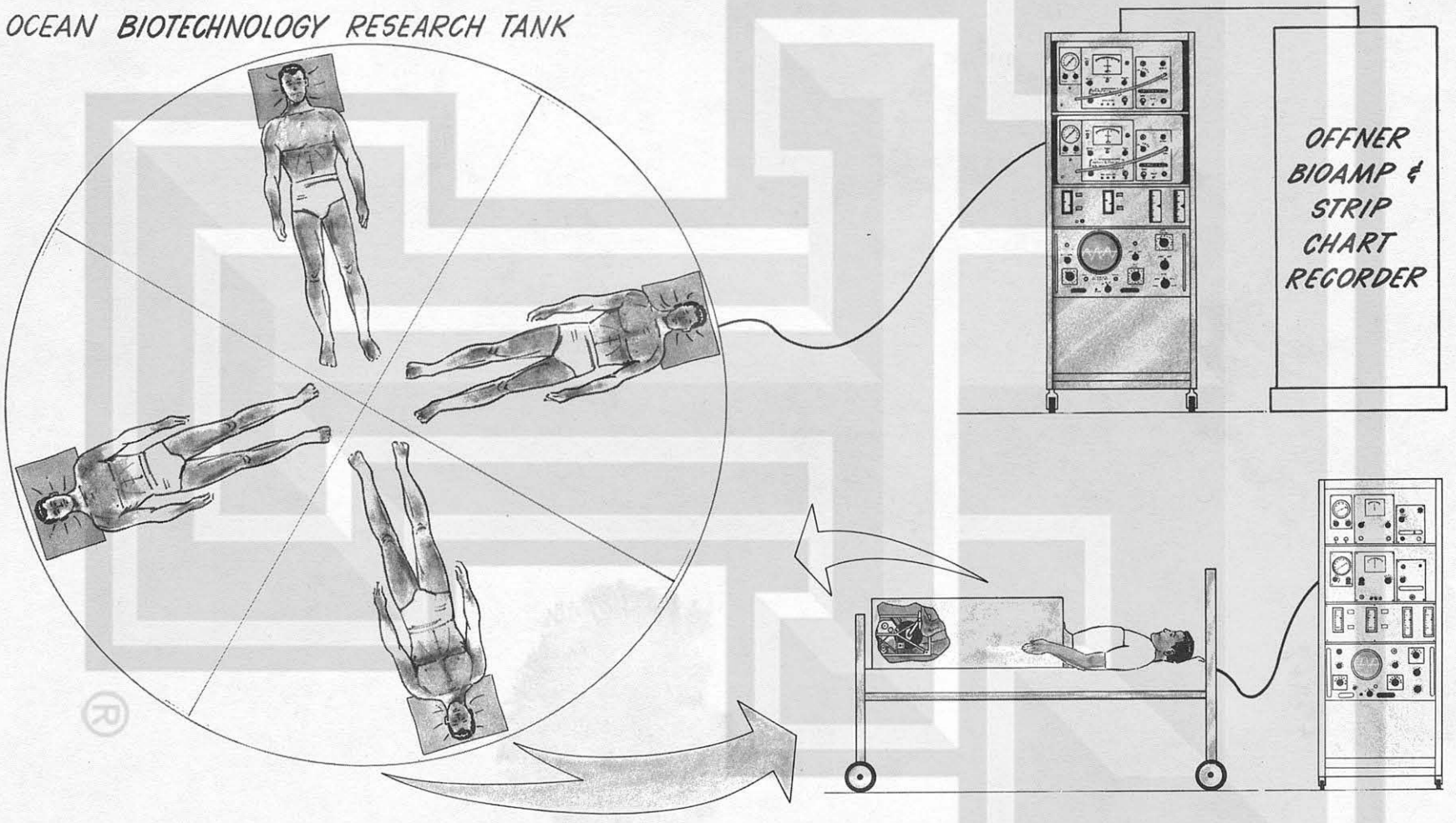


Figure 3

Figure 4

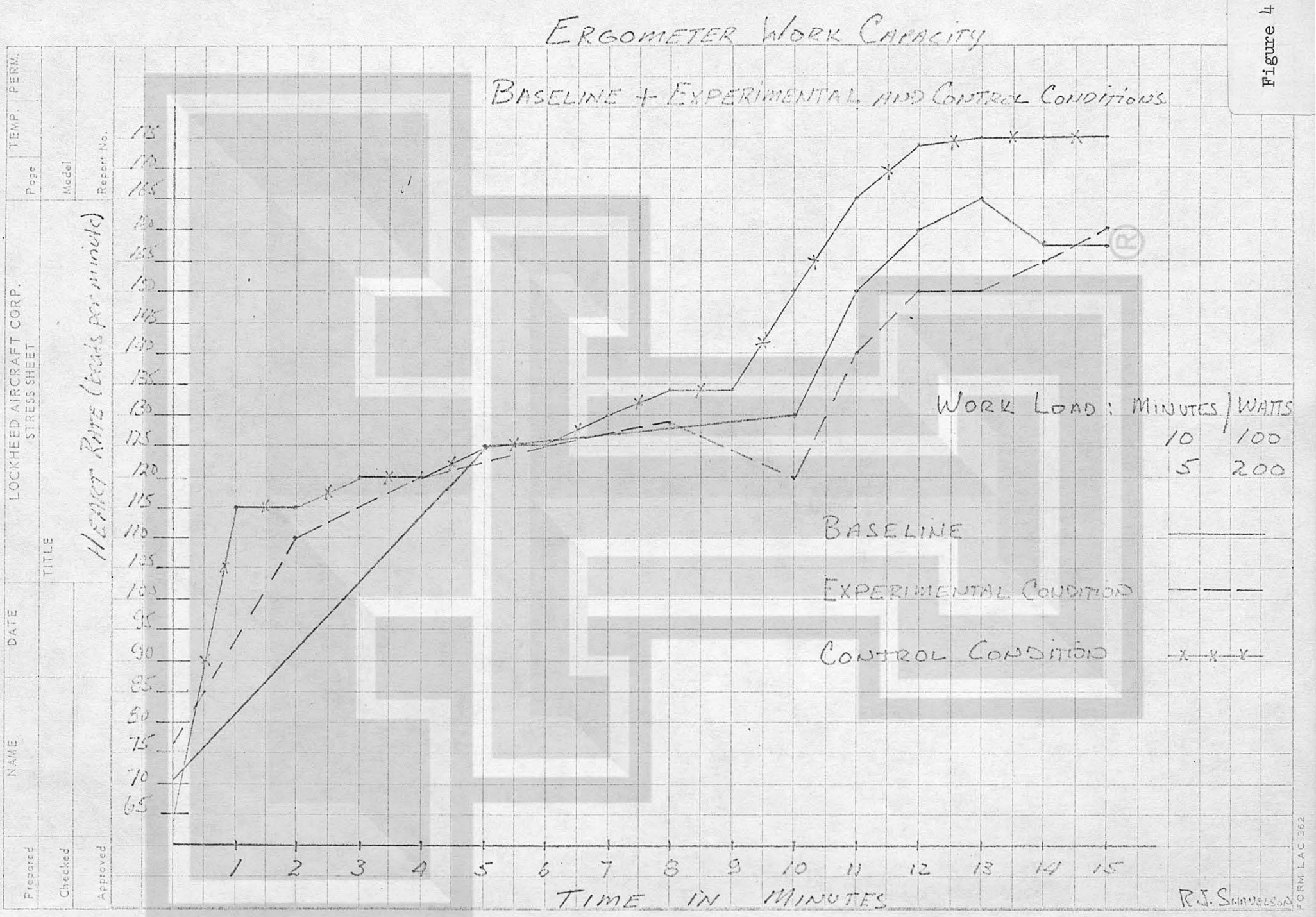


FIGURE 4