ADENOSINE CORONARY DILATION AS INFLUENCED BY PERFUSATE HYDROGEN ION ACTIVITY AND THEOPHYLLINE

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Gary F. Merrill

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J M Dabney
Major professor

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ABSTRACT

ADENOSINE CORONARY DILATION AS INFLUENCED BY PERFUSATE HYDROGEN ION ACTIVITY AND THEOPHYLLINE

By

Gary F. Merrill

Adenosine has been proposed as a mediator of reactive hyperemia and hypoxic coronary dilation. This hypothesis is based on the finding that myocardial levels of adenosine increase during myocardial ischemia and hypoxia. However, while theophylline, a competitive antagonist of adenosine, attenuates the coronary dilator response to exogenously administered adenosine, it fails to affect greatly the magnitudes of reactive and hypoxic hyperemias. The hydrogen ion concentration also increases during myocardial ischemia and hypoxia. Therefore, an objective of the present study was to determine whether or not the inability of theophylline to attenuate reactive hyperemia is related to an interaction of hydrogen ion with theophylline and/or adenosine.

An isolated perfused guinea pig heart was used in all experiments. The coronary vascular bed was perfused at constant pressure (65 cm H_2O) and temperature (38°C) from a reservoir with a modified Ringer's solution gassed with

95% O_2-5% CO_2 (pH 7.42 $\pm$ 0.01). Coronary inflow was measured with an electromagnetic flowmeter and coronary outflow with a graduated cylinder and stopwatch. Adenosine and theophylline were added volumetrically to the reservoir, and pH was varied by altering the PCO_2 of the perfusate.

Coronary flow increased as a function of the adenosine concentration over the range of 10^{-8} to 10^{-6}M. When the perfusate pH was reduced to 7.36 and 7.20, coronary flow increased but the level achieved with 10^{-6}M adenosine was the same as at pH 7.43. However, at pH 6.89 the vasodilator activity of adenosine appeared to be enhanced, as it was when hearts were initially perfused at pH 7.20 following stabilization. When perfusate pH was subsequently increased to pH 7.69, coronary flow failed to change and the vasodilator activity of adenosine appeared to be suppressed. At pH 7.20, the vasodilator activity of $8x10^{-7}$M adenosine was sustained for at least 30 minutes; a finding previously observed at a perfusate of pH 7.4. If adenosine is more active in the presence of acidosis, this could in part explain why theophylline fails to greatly modify reactive and hypoxic hyperemias.

Theophylline was without effect on coronary flow and heart rate in concentrations up to 10^{-4}M (at higher concentrations coronary flow and heart rate increased).

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The vasodilation produced by $8x10^{-7}M$ adenosine was attenuated by theophylline ($10^{-4}M$) at both pH 7.42 and 7.20.

Theophylline, $5x10^{-5}M$, was essentially without effect on the reactive hyperemia seen following 30 seconds of coronary inflow occlusion. Since the potassium and hydrogen ions have also been suggested as mediators of hypoxic and ischemic dilation and of autoregulation, ouabain ($1.4x10^{-7}M$), a blocker of potassium vasodilation, and alkalosis (perfusate pH 7.69) were combined with the theophylline in an attempt to modify these manifestations of local regulation by minimizing the contribution of potassium and hydrogen ions and adenosine. Hypoxic dilation was unaffected. Both the volume and duration of hyperemic flow were reduced somewhat but failed to return to control after normalizing the perfusate. Autoregulation, while modified, still occurred.

These studies suggest that an increase in hydrogen ion concentration enhances adenosine's coronary action and a decrease in hydrogen ion concentration diminishes adenosine's coronary action. They also show that reducing the perfusate pH has little effect on theophylline's ability to inhibit adenosine induced coronary dilation. Further, adenosine, at a low pH, has the ability to maintain coronary dilation over an extended period of time.

ADENOSINE CORONARY DILATION AS INFLUENCED BY PERFUSATE HYDROGEN ION ACTIVITY AND THEOPHYLLINE

By

Gary F. Merrill

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
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DOCTOR OF PHILOSOPHY

Department of Physiology

1975

DEDICATION

To my wife Marlene
for all that she is.

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I wish to express appreciation to the members of my guidance committee: Drs. J. M. Dabney, F. J. Haddy, J. B. Scott, B. H. Selleck, and J. L. Stickney for the time and effort they have spent in my behalf. I have benefited greatly from the perception and persistence shown by each.

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INTRODUCTION AND SURVEY OF THE LITERATURE

I. Local Blood Flow Regulation: Definition, Forms of and Chemical Participants

The phrase 'local regulation of blood flow' refers to that regulation which is intrinsic to the organ. Specifically excluded is regulation secondary to remote influences which alter vasoconstrictor or vasodilator nerve activity or the concentrations of vasoactive substances in inflowing blood (Haddy and Scott, in press). The main forms of local regulation are: 1) autoregulation--the ability of an organ to maintain a relatively constant blood flow despite changes in arterial perfusion pressure, 2) active hyperemia--an increase in blood flow above control levels in response to increased metabolic activity, and 3) reactive hyperemia--a transient increase in flow to a level greater than control following a period of reduced flow.

One hypothesis for the local regulation of blood flow is the metabolic hypothesis, which proposes in its simplest form that changes in metabolism or blood flow are accompanied by alterations in the concentrations of oxygen and vasodilator metabolites in the tissue fluids. Such alterations result in active vasomotion which adjusts flow to a

level more appropriate to the metabolic rate (41).

Gaskell (34) was the first to report that artificially exercising skeletal muscle by electrically stimulating the motor nerves to the muscle, produced an increase in blood flow through the active muscle. Since Gaskell's report, numerous others (31,43,54,77,88) have verified that skeletal muscle has the capacity to adjust its blood flow to meet metabolic needs, and still others have expanded the findings to most of the major vascular beds (6,7,17,25,30,32,42,46, 52,75,90,93). Amongst the vascular beds able to regulate their blood flow is the coronary bed. For example, studying the dog heart-lung preparation, Hilton and Eichholtz (47) reported that coronary vessels are extremely susceptible to changes in blood oxygen tension, a fall of oxygen saturation below 20 per cent causing maximal dilation. Cross et al. (14) studied autoregulation in isolated heart preparations and in intact, innervated dog hearts, and demonstrated this organ's ability to maintain a relatively constant flow over a broad range of arterial pressures. Driscol, Moir and Eckstein (20) studied coronary autoregulation in the anesthetized dog and reported that coronary flow responses to changes in perfusion pressure are not significantly affected by collateral flow. They found that pressure-flow responses (examined by perfusing one of the first major branches of the left circumflex coronary) were

the same with and without perfusing simultaneously the left common coronary artery at the same pressure, i.e., the absence of a pressure gradient between the branch of the left circumflex and its collaterals did not effect the coronaries ability to autoregulate. More recently Bunger et al. (11) using an isolated guinea pig heart found that in the presence of pyruvate (2.0 mM) plus glucose (5.5 mM) this preparation displayed typical autoregulatory pressure-flow responses over the pressure range of 20-90 cm H_2O. Reactive dilation, following release of occlusion, was also observed and was found to be typical of reactive hyperemic responses reported for the in vivo heart.

A. Involvement of Oxygen in Local Flow Regulation

It is evident that those conditions which reduce tissue oxygen tension via increased oxygen utilization and reduced oxygen delivery favor an increase in blood flow to meet the metabolic needs of the tissue (5,12,13,24,26,39,48,58). That oxygen is directly or indirectly involved in exercise hyperemia (51,61,70), reactive hyperemia (61,65,70) and autoregulation (53,54) in cardiac and skeletal muscle is well documented. Studying the effect of blood oxygen saturation on autoregulation in the dog hindlimb, Guyton et al. (39) found that blood flow progressively increased as the per cent oxygen saturation of arterial blood is decreased.

Flow increased on the average of two and one-half times as oxygen saturation was decreased from 100 per cent to 30 per cent. Carrier and co-workers (12) using short segments of isolated dog femoral artery, and Detar and Bohr (19) studying helical strips of rabbit aortas, found that reducing the oxygen tension of perfusing/bathing solutions results in relaxation. Daugherty and his associates (16) demonstrated a reduction in coronary resistance when the oxygen tension of blood perfusing the coronaries was decreased below 40 mm Hg. The effect of oxygen content on coronary blood flow was studied by Guz et al. (40) while perfusing the isolated rabbit heart with a hemoglobin solution. They found that reducing the arterial oxygen content by diluting the perfusate with Ringer-Locke's solution increased coronary flow; whereas, increasing the arterial oxygen content decreased coronary flow.

B. Participation of Hydrogen Ion and Carbon Dioxide in Local Flow Regulation

Several studies have shown that local exposure of blood to increased PCO_2 or local administration of acid, reduces resistance to blood flow through skeletal muscle (16,37), cerebral (29,35) and coronary (16) vascular beds, while reduction of the local carbon dioxide tension or administration of alkali, increases resistance to flow through the forelimb (16,66), brain (29,35), and heart (16,38,63).

Investigating the effect of hydrogen ion concentration on coronary flow in an isolated rabbit heart, Smith and co-workers (86) found that a decrease in perfusate pH from 7.68 to 7.38 increased flow 7 per cent. When pH was further reduced to 7.0, coronary flow was augmented 80 per cent relative to that at pH 7.68. McElroy, Gerdes and Brown (63) reported, from a study using an isolated, perfused guinea pig heart, that only when changes in HCO_3^- and PCO_2 produced alterations in pH was coronary flow affected. In these and similar studies the effects of rather large changes in pH were studied, while more direct evidence (18,37,61,81,82) indicates that local regulation may be accompanied by marked changes in resistance with very small or no change in venous blood pH occuring. Hence, factors in addition to hydrogen ion and carbon dioxide must play a part in local flow regulation.

C. Participation of Metabolites Other Than O_2, CO_2 and pH

Bioassay and chemical analysis of venous blood support the argument that metabolites other than oxygen, hydrogen ion and carbon dioxide are involved in local regulation of blood flow (41,75,81,82). Scott et al. (85) reported that chemical analysis of venous effluent blood from an exercising dog gracilis muscle showed an elevation in potassium ion concentration. However, the importance of potassium in

the regulation of coronary flow is questionable, i.e., hyperkalemia may transiently increase coronary flow (64) but has been shown to produce an increase in coronary resistance (60). Hypokalemia decreases coronary flow (44).

Other chemicals such as lactate, pyruvate, inorganic phosphate and Krebs cycle intermediate metabolites have been shown to increase in skeletal muscle venous blood with exercise and reactive hyperemia (41,42) and during cardiac hypoxia (5). Although many of these factors are capable of eliciting some degree of vasodilation in skeletal and cardiac vasculature, most fail to meet the criteria necessary for a true physiological mediator of blood flow (41).

D. Simultaneous Contribution of Oxygen and Hydrogen Ion

Several investigators have attempted to assess the simultaneous contribution of oxygen lack and increased hydrogen ion to the regulation of local blood flow. In one study, Scott et al. (85) selectively lowered the oxygen tension of blood flowing into the resting hindlimb to a level which produced a low venous oxygen tension. The subsequent fall in vascular resistance was noted. Arterial inflow oxygen was then selectively increased to a high level and exercise initiated. Venous oxygen tension did not fall to the level seen during hypoxia but vascular resistance fell to a level which was lower than that during

hypoxia alone, thus demonstrating that chemicals other than oxygen were producing vasodilation. In another study, Stowe and co-workers (89) pumped venous blood from a dog gracilis muscle through a hollow fiber gas-exchange permeator into the artery of an assay gracilis. During electrical stimulation of the regulatory muscle's motor nerve, calculated resistance of both muscles decreased. PO_2 and pH of the blood perfusing the assay muscles both decreased. When PO_2 or pH were individually corrected to control values, calculated resistance returned toward but did not reach control values. However, if both PO_2 and pH of blood flowing into the assay muscle were successfully corrected to pre-exercise levels, dilation in the assay muscle disappeared.

II. Adenosine Hypothesis for the Local Regulation of Coronary Blood Flow

Changes in regional myocardial metabolism are associated with alterations in coronary blood flow which are immediate and marked in effect (68). There is loss of stored ATP (68,78,80) and creatine phosphate (68) within seconds of an occlusion. Hydrolysis of stored triglyceride can result from activation of myocardial lipase (67). In addition it has been shown more recently that associated with myocardial hypoxia, is the release of adenosine (7,76, 78,79), a major degradative product of ATP.

In 1963, some 40 years after Drury's (21,22) pioneering demonstration that adenosine is a potent coronary dilator, Berne (5) introduced the current adenosine hypothesis for the metabolic regulation of coronary blood flow in which he proposed a mechanism whereby coronary flow increases during hypoxia. Berne suggested that any condition which tends to lower cardiac tissue oxygen tension, e.g., increased metabolic activity, reduced coronary flow, and/or hypoxemia would result, via net adenine nucleotide degradation, in the enhanced production and release of adenosine from the myocardial cell (Figures 1 and 2). Adenosine could subsequently pass through the interstitial fluids and act upon the coronary resistance vessels to dilate them. Since 1963, much evidence has accumulated in support of Berne's "adenosine hypothesis" (6,7,8,15,58,78,84); however, for some time this hypothesis was viewed with skepticism because investigation failed to reveal adenosine in the effluent perfusate of isolated (5,49,76) or intact heart preparations (5).

The failure to find adenosine in the venous effluent prompted the undertaking of studies aimed at determining if the proper catabolic enzymes were present in the myocardium whereby adenine nucleotides could be hydrolyzed to adenosine. In one experiment, using pig heart muscle extracts, Goldthwait (36) reported that adenosine kinase, adenosine

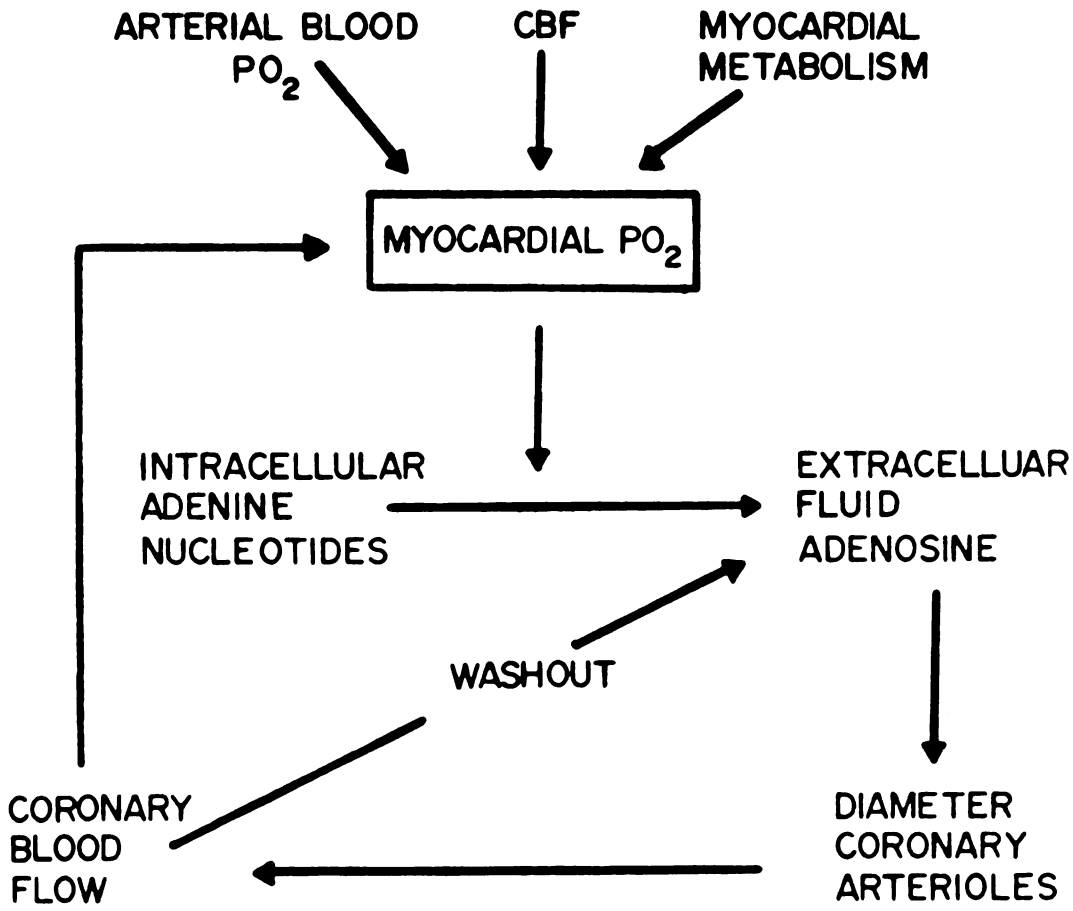

Figure 1: Schema illustrating adenosine hypothesis for regulation of coronary blood flow.

(Reproduced from Am. J. Physiol. 204: 317, 1963, by permission)

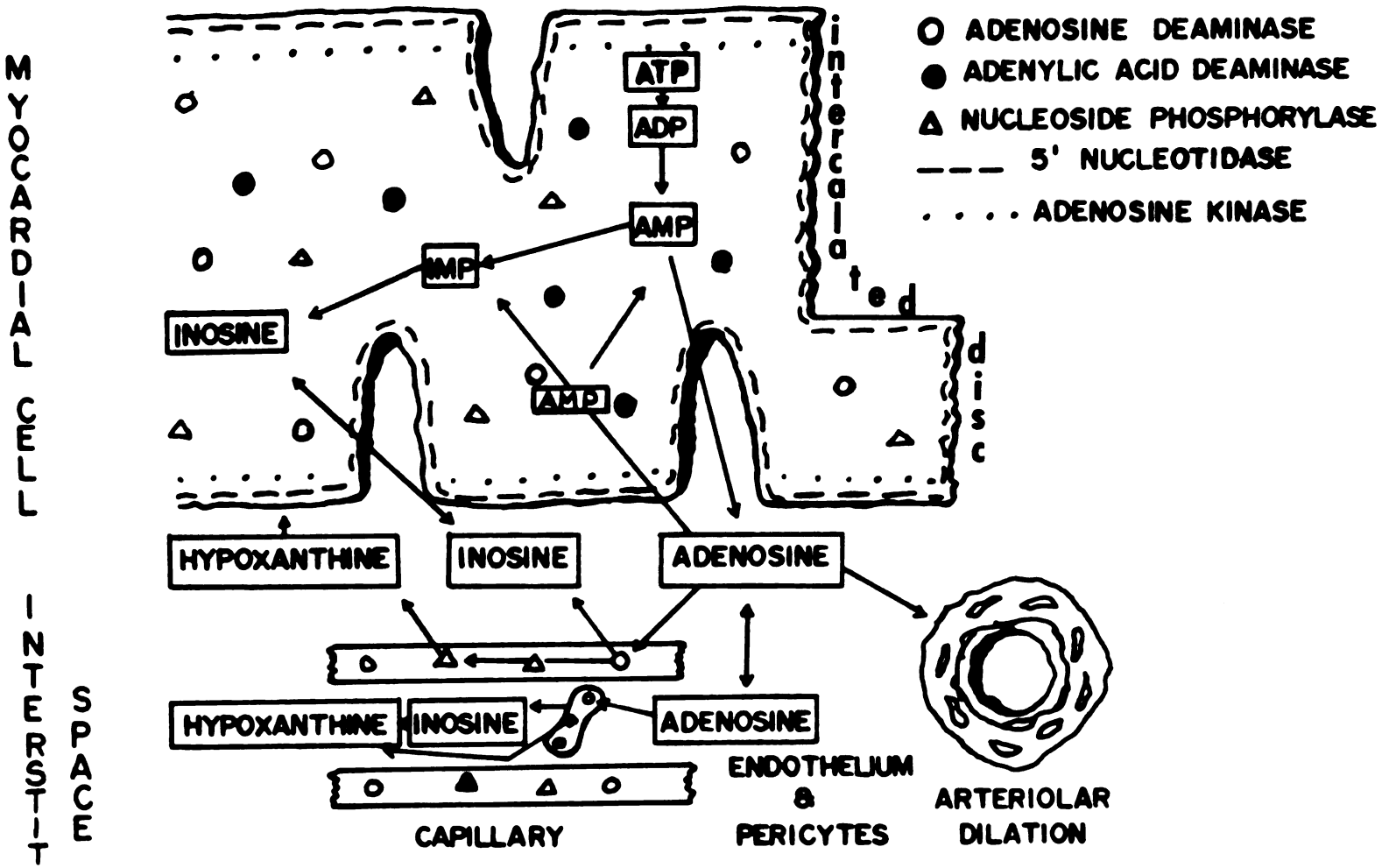

Figure 2: Schematic diagram of myocardial tissue illustrating formation, fate, and site of action of adenosine coming from intracellular ATP.

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deaminase, nucleoside phosphorylase and adenylic acid deaminase are all present in this tissue. In addition, nonspecific phosphatases (45), as well as specific 5' nucleotidases (45) which result in dephosphorylation of adenylic acid have been described. Further, adenosine conversion by the enzyme nucleoside phosphorylase (57) to the free base and a sugar derivative has been shown. Finally, adenosine can be converted to inosine by the enzyme adenosine deaminase.

Later it was reported that in isolated heart preparations adenosine deaminase and nucleoside phosphorylase were leached out of the perfused heart before inactivation of these enzymes could be accomplished (76), thus converting any adenosine which might have been present to inosine (5,76) and to the free base, adenine (36). Other experiments with in vivo dog hearts revealed that the time required to collect coronary sinus blood and separate cells and plasma was sufficient for complete destruction of any adenosine which might have been present (5). Thus it was apparent that adenosine is formed inside the myocardial cell but could be rapidly inactivated once out of the cell.

Not only must the enzymes for adenine nucleotide degradation to adenosine be present in the myocardial cell, it must be shown that adenosine is capable of leaving the cell and reaching the site of dilatory action before being

inactivated. In a group of experiments in which metabolism of purine derivatives in an isolated cat heart was studied, Jacob and Berne (49) considered the question of adenosine release from the cell. They reported that adenosine, added to perfusate and collected after one passage through the cat heart, was converted to inosine and hypoxanthine. When hearts were perfused with solution containing adenosine-8-C^{14} approximately 50 per cent of the adenosine entered the myocardial cells where it was trapped as adenine nucleotide; the remainder was recovered in the perfusate as inosine and hypoxanthine.

Subsequent reports appeared in which it was found that pretreatment of hearts with 8-azaguanine, an inhibitor of adenosine deaminase (5,76) or with uncouplers of oxidative phosphorylation such as dinitrophenol (49) and bishydroxycoumarin (76), allowed adenosine's presence in venous effluent blood (5,76,78).

A. Support for and Opposition to Adenosine's Involvement in Hypoxic Coronary Dilation

Early in the genesis of the adenosine hypothesis, Berne (5) reported that from three to 27 times more inosine and hypoxanthine were released from the heart during myocardial hypoxia than were required to double the coronary blood flow when infused as adenosine into the left coronary artery. This report has since been followed by a deluge of

reports attempting to substantiate or refute the claim for a direct role by adenosine in mediating hypoxia-induced changes in coronary blood flow. Berne (5) studied the effects of recirculating hypoxic Tyrode's solution through isolated cat hearts and found that hypoxanthine and inosine appeared in the perfusate. Reinstitution of oxygenated Tyrode's solution produced a decrease in the quantity of these compounds released from the heart. In other studies of hypoxia and coronary flow, Katori and Berne (58) perfused isolated cat hearts and isolated guinea pig hearts with solution equilibrated with graded concentrations of oxygen (95, 65, 48, 30, 20, 10, and 0 per cent). In each hypoxic experimental period, hearts were perfused with 200 ml of solution which was subsequently collected in flasks immersed in ice. Just prior to each collection period, 8-azaguanine was infused into the aortic cannula at a rate calculated to give a final concentration of 10^{-3}M. Both anoxia and severe hypoxia in the guinea pig hearts produced similar results regarding the release of adenosine, inosine and hypoxanthine. The magnitude of the sum of the released adenosine, inosine and hypoxanthine was greater however during anoxia. During pre- and posthypoxic control periods, small quantities of hypoxanthine and inosine were detected in the perfusate. Although these experiments (5,58) revealed the presence of inosine and hypoxanthine in

coronary sinus blood, neither of these was present in arterial blood, suggesting that their site of origin was in the myocardium and that partial asphyxia did not produce a release of such compounds from other body tissues in quantities sufficient to be detected in arterial blood.

Richman and Wyborny (76) investigated adenine nucleotide degradation in response to hypoxia by analyzing heart tissue extracts and effluent from an isolated rabbit heart. They reported that only in the presence of 8-azaguanine (1-5 mM) were detectable quantities of adenosine present in the effluent perfusate and in hypoxic cardiac tissue. In their study the effects of adding to the perfusate bishydroxycoúmarin ($5x10^{-3}$M) were also examined. It was found that six minutes after initiation of perfusate containing this agent, ADP, AMP, adenosine, inosine and IMP concentrations in tissue extracts and in effluent perfusate were significantly elevated while ATP concentration decreased.

In a more recent paper, Rubio and associates (80) reported that as the PO_2 of Krebs-Henseleit solution was reduced, per cent change in coronary flow, tissue adenosine, and the rate of release of adenosine into the perfusate of an isolated guinea pig heart increased in a parallel fashion at oxygen tensions less than that at 60 per cent O_2.

Based upon the findings that adenosine is released during myocardial hypoxia, and that coronary dilation by

exogenous adenosine can be blocked with theophylline/aminophylline (1,2,9,55,56,91) and potentiated by lidoflazine and/or dipyridamole (27,28,62), Afonso (1,2) and others (9,91) reasoned that if hypoxic coronary dilation could be blocked with aminophylline (chosen over theophylline because of its greater solubility in water, but with coronary vasoactivity that is quantitatively similar to that produced by theophylline) added support would be given the adenosine hypothesis. Afonso et al. (2) used mongrel dogs to study the possible influence of aminophylline on the coronary dilation produced in response to hypoxia. After control measurements of heart rate and coronary sinus blood flow were made, animals were ventilated on a mixture of 5 or 8 per cent oxygen in air. Results from these experiments indicated that the increase in coronary blood flow, before and after aminophylline (7.5 mg/kg) were of the same magnitude. In an attempt to explain these findings that would be consistent with the adenosine hypothesis, Afonso suggested that perhaps the degree of hypoxia studied produced maximal coronary dilation. This possibility was ruled out by the subsequent demonstration that the coronary bed would be further dilated by adenosine in the presence of hypoxia.

Anesthetized cats were used by Wadsworth (91) in studies which confirm the findings of Afonso (2) that

aminophylline does not attenuate coronary dilation caused by hypoxia. When cats were ventilated with a mixture of oxygen and nitrogen (7.8 per cent oxygen) the arterial PO_2 fell from 91 mm Hg to 23 mm Hg, coronary blood flow rose, and calculated myocardial vascular resistance fell. Intravenous injection of 10 mg/kg aminophylline had no measurable effect on the hypoxic coronary blood flow. Wadsworth concluded that adenosine might not be involved in the regulation of coronary blood flow during hypoxia.

B. Support for and Opposition to Adenosine's Involvement in Coronary Reactive Hyperemia

A second form of local flow hypothesized by Berne to be mediated by adenosine is coronary reactive hyperemia. Results from investigation testing this claim have been nonuniform partly because this form of local flow regulation has been characterized variously (9,15,56) by quantitating the 1) time interval between the beginning and the maximum of reactive hyperemia, 2) volume ratio, i.e., the ratio between excess flow: the total volume of flow collected between release of an occlusion and the point at which the hyperemic flow declines to the preocclusion flow level, and flow deficit: the total volume of flow that would have occurred during the period of inflow occlusion (56), 3) duration of the hyperemia (15,56), 4) total volume of coronary blood flow from the onset of reactive

hyperemia to the point where flow has returned 50 per cent toward control flow (15), 5) magnitude of the hyperemic response (peak flow) (15), 6) elapsed time from release of occlusion to the point at which flow was returned 50 per cent toward control, and 7) per cent repayment of flow debt (27,28).

Using anesthetized mongrel dogs, Rubio et al. (78) undertook experiments to determine whether adenosine is released from the myocardium in response to moderate degrees of myocardial ischemia and whether the quantities released could account for the degree of coronary vasodilation observed. Samples of arterial blood and coronary sinus blood were taken simultaneously prior to occlusion and immediately after occlusion and separated plasma samples were analyzed for purine derivatives. Adenosine (13 nmoles/100 ml plasma) was found in coronary sinus blood collected during reactive hyperemia but was absent from arterial and sinus blood collected before occlusion. These workers concluded that if all the adenosine is in the extracellular space, a minimum concentration of 75 nmoles/100 ml of extracellular water would be reached. Further, they reported that infusion of adenosine (56 nmoles/100 ml) into arterial blood produces maximum coronary dilation.

Following 30 or 120 second occlusions of the left anterior descending artery in the anesthetized cat,

Wadsworth (91) observed a peak hyperemic flow two to three times that of control flow. In this group of animals, he found that 15 minutes after the injection of aminophylline (7 mg/kg), the period of reactive hyperemia (measured from the moment of release of the coronary snare until flow was returned 50 per cent toward control) was noticeably reduced while the magnitude of the hyperemic response was only slightly altered.

Juhran and co-workers (56) completed a detailed study of reactive hyperemia in the conscious dog and assessed alterations of control responses produced by pretreating the animals with theophylline, dipyridamole or lidoflazine. These investigators found that when the period of inflow occlusion exceeded 30 seconds threshold doses of dipyridamole (0.5 mg/kg i.v.), although without an effect on the magnitude of reactive hyperemia, did potentiate the duration and volume ratio of the response. Further examination revealed that the injection of theophylline (8 mg/kg i.v.) eliminated the dipyridamole-induced potentiation of reactive hyperemia. This suggested to them that notable amounts of adenosine are liberated only after complete occlusion of coronary vessels for 30 seconds. In subsequent experiments the effects of theophylline on the time course of reactive hyperemia was examined and it was discovered that the duration of reactive hyperemia following 15, 30, 60 and 90 second occlusions was reduced. Additionally, the rise in

coronary flow upon release of occlusion was markedly accelerated in the 60 and 90 second trials, suggesting the probable accumulation of adenosine during occlusion. The findings of the above experiments (56) support adenosine's involvement in reactive hyperemia if occlusion time exceeds 30 seconds and if the duration of reactive hyperemia is the criterion used to quantitate the response.

Curnish, Berne and Rubio (15) examined the duration, volume and peak flow responses to inflow occlusions before and after aminophylline (5 or 10 mg/0.2 ml). They reported that aminophylline produced a decrease of approximately 42 per cent in the volume of hyperemic flow, and 31 per cent in the duration of the response following brief (10 and 20 seconds) occlusions. Peak flow was unaffected by aminophylline. When these results were subsequently compared with results obtained during aminophylline blockade of the coronary response to 4 μg of intra-arterial adenosine, they were found to be similar.

Eikens and Wilcken (27,28) recently challenged the claim that with long periods of occlusion adenosine levels reach those which escape (15) theophylline/aminophylline attenuation during the subsequent reactive hyperemia. They demonstrated that inflow occlusions of four, eight and sixty seconds in dogs were not affected by either aminophylline or dipyridamole. When inflow was occluded for 60 seconds,

then released, there was a three and a half times increase in flow above control in the absence of aminophylline. During infusion of aminophylline (200 μg/min), the corresponding increase in flow upon release of occlusion was still three and a half times above control. Similar results were gathered when these investigators studied dipyridamole's effects on coronary reactive hyperemia, i.e., responses were similar in the presence and absence of this agent. Eikens and Wilcken concluded that adenosine release into the extracellular space is unlikely to be essential for the adaptation of coronary blood flow to the metabolic needs of the myocardium under physiological conditions.

Other results which fail to support the involvement of adenosine in reactive hyperemia come from the work of Bittar and Pauly (9), who evaluated reactive hyperemia following inflow occlusions of 30, 60 and 120 seconds in the presence or absence of aminophylline or lidoflazine. In a typical experiment they found that the total volume of hyperemic flow following release of a 30 second occlusion was unaffected by either aminophylline (100 mg i.v.) or lidoflazine (0.4 mg/ml i.a.). When increasing the occlusion time to 60 or 120 seconds there was still no effect by either agent. Bittar and Pauly also concluded that reactive hyperemia in the dog is not due solely to the local release of adenosine.

Finally, Juhran and Dietmann (55) reported that theophylline (16 mg/kg i.v.) failed to influence the magnitude of reactive hyperemia following a 15 second occlusion in the conscious dog.

III. A Statement of Objectives

The major objective of the following study was to clarify the current paradox that theophylline attenuates coronary dilation by exogenous adenosine but does not consistently attenuate reactive/hypoxic coronary hyperemias. It seemed possible that an interaction between hydrogen ion and adenosine might enhance adenosine's coronary action during reactive hyperemia and hypoxic coronary dilation thereby decreasing the ability of theophylline to block these responses. Alternatively, it was considered that theophylline's adenosine-attenuating ability might be minimized by an increased hydrogen ion activity. Specifically, the effect of altering hydrogen ion concentration on adenosine's ability to dilate the coronary vascular bed was studied in an effort to answer the question: Is adenosine's capacity to dilate the coronary vasculature modified by a change in pH? Furthermore, the effect of altering hydrogen ion concentration on theophylline's ability to attenuate adenosine coronary dilation was studied.

During the course of the study it appeared useful to consider other questions: 1) Does the dilating action of exogenous adenosine persist or does it wane with time? 2) Does altering the routine sequence of perfusing hearts affect the dilating action of adenosine, or the blocking capacity of theophylline? 3) Is reactive hyperemia and/or hypoxic dilation affected by concurrent administration of theophylline, ouabain and alkalosis? 4) Can the coronary vasculature still autoregulate flow in the presence of concurrent theophylline, ouabain and alkalosis?

EXPERIMENTAL METHODS

The isolated perfused guinea pig heart was used to study the current controversy attending Berne's adenosine hypothesis for the regulation of coronary blood flow. This particular heart model was selected based on the recent demonstration by Bunger *et al.* (10,11) that this preparation possesses coronary responsiveness and reactivity which is much like that of *in vivo* heart preparations. It is stable for more than 90 minutes with respect to coronary flow, heart rate, left ventricular pressure, dp/dt, oxygen consumption, and myocardial high energy phosphate levels. Further, the changes in coronary flow induced by alterations of perfusion pressure, ischemia and hypoxia resemble those seen under *in vivo* conditions. The perfusate was fortified with 2.0 mM pyruvate as described by Bunger *et al.* (10,11). These workers attributed the *in vivo*-like features of their isolated guinea pig heart to the addition of pyruvate.

I. Preparation of the Isolated Guinea Pig Heart (Figure 3)

Hartley strain guinea pigs (Cannaught Laboratories, Willowspring, Ontario) of either sex fed *ad libitum* and

weighing 290-320 grams were stunned with a blow on the head and secured to a guinea pig table. A bilateral thoracotomy was performed to expose the heart. The pericardium was removed and the heart was arrested by superfusing with ice-cold saline. The ascending aorta was isolated and freed from periaortic fat and connective tissue and two silk threads (size 4-0) were passed beneath the aorta. An incision was made in the dorsal wall of the aorta and the tip of a polyethylene cannula (PE 240 i.d. 0.066 in.), connected to a removable 12 inch section of rubber latex tubing (1/8 inch i.d.), was inserted and firmly tied in position. Perfusion of the total coronary vasculature of the in situ heart was begun via the aorta with Krebs-Ringer bicarbonate solution containing (mM): glucose 5.5, pyruvate 2.0, NaCl 127.5, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, and $NaHCO_3$ 24.9 and equilibrated with 5% CO_2-95% O_2 (pH 7.42 $\pm$ 0.01, 38°C). Care was taken to ensure that the cannula tip did not damage the aortic valve. Gentle squeezing of hearts prevented air from entering aortas when hearts were subsequently transferred to a non-recirculating perfusion system (65 cm H_2O perfusion pressure; Figure 3). An effort was made from the time of sacrifice, to complete the heart set-up as rapidly as possible, thus sparing unnecessary and prolonged ischemia/anoxia. The sacrifice-to-cannulation time usually required three to four minutes. Hearts were

Figure 3. Diagram of the non-recirculating perfusion system used in the isolated, perfused guinea pig heart experiments. Perfusion pressure, 65 cm H_2O, Temp. 38°C.

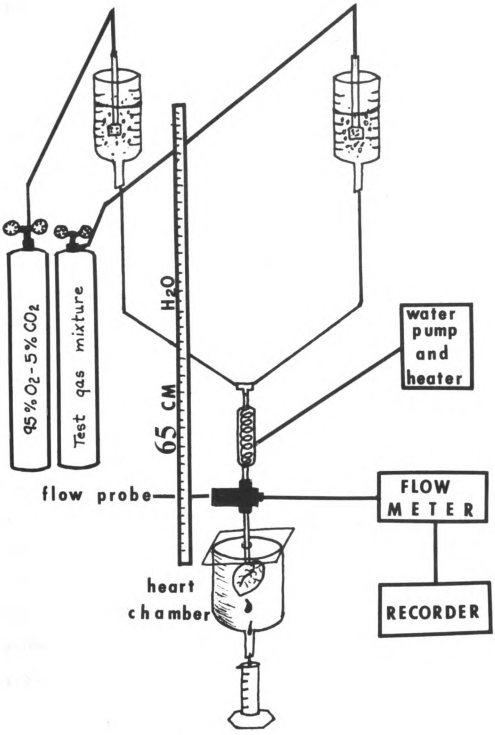

95% O2-5% CO2
Test gas mixture
H2O
65 CM
water pump and heater
flow probe
FLOW METER
heart chamber
RECORDER

FIGURE 3

subsequently cut free from surrounding fatty and connective tissue and the section of rubber tubing was removed. To minimize the effects of transmural pressure changes on the caliber of the blood vessels, right and left heart chambers were modified to prevent accumulation of perfusate. Modification was achieved by making an incision (102 mm) in the apex of the right ventricle (sparing visible coronary vessels), and the left atrium and the anteromedian area of the right atrium were removed. Care was taken to avoid damage to the region of the sinoatrial node (posterolateral aspect of the right atrial appendage at its junction with the superior vena cava and coronary vessels) (3). The mitral valve was cut following removal of the left atrial appendage.

A glass distillation column (vol. 3.5 ml) was located in the perfusion circuit 8-10 inches proximal to the heart and served as the final heat source for maintaining the temperature of the perfusate. The column, heart chamber (vol. 225 ml) and perfusate reservoirs (vol. 500 ml) were jacketed and warmed by circulating distilled water (38°C) with a Haake (Model E12, Haake Instruments, Inc., Saddle Brook, N.J.) variable heater pump.

II. Experimental Equipment and Calibration

Retrograde aortic inflow (assumed to equal coronary inflow) was continuously monitored with a Biotronex BL-610 Pulsed-Logic flowmeter (Biotronex Laboratory, Inc., Silver Spring, Md.). An extracorporeal flcw transducer (BLC-2024-F10, 3/8 in. i.d.) was placed in the perfusion circuit approximately 10-15 cm proximal to the isolated heart. The flowmeter was connected to a Hewlett Packard 350-1000 DC preamplifier, and the flow pattern, either phasic or mean, was recorded on a Sanborn (model 296) direct-writing paper recorder. The preamplifier was balanced and the flow transducer was calibrated at the beginning of each day's experiments. A transducer calibration curve, in which recorder stylus deflection (mm paper) was plotted against coronary flow (ml/min), was occasionally constructed and a ratio of approximately 1:1 was always established. The calibration curve allowed us to follow the electronic drift of the flowmeter and to make corrections whenever necessary. Electronic drifting was assessed by checking the equality of mechanical zero with the fixed zero reference (baseline zero). Three such checks were routinely made during the course of an experiment: 1) immediately prior to cannulating an aorta, 2) at the time of inflow occlusion when examining reactive dilation, and 3) just prior to the termination of each experiment. Mechanical zero was determined by

occluding coronary inflow when the flowmeter output selector dial was turned to the "flow" position. Timed collections of flow using a stopwatch and graduated cylinder were taken during every experiment as a second means of estimating electronic drift of the flowmeter. Electronic drift was variable, often amounting to 5-15 per cent/hr.

III. Stabilization and Tests of Coronary Responsiveness

A. Stabilization

After the heart was attached to the non-recirculating perfusion system, a period of 20-30 minutes was allowed for heart rate to stabilize and the high coronary flow to subside. During this time any arrhythmia generally disappeared. At the end of the stabilization period, hearts that did not beat rhythmically at a rate in excess of 200/min and whose coronary flow was not stable were discarded.

B. Coronary Responsiveness

Two tests were used to assess the responsiveness of the coronary bed: 1) The inflow cannula proximal to the flow transducer was occluded for 30 seconds. Upon release the postocclusion reactive dilation in acceptable preparations produced a peak flow two to three times greater than control, which returned to control within 30-40 seconds. 2) Five minutes later, a 250 μg bolus of adenosine was

rapidly injected into the aortic cannula 3 cm proximal to the heart. Eight to twelve cardiac cycles later, the heart stopped in diastole, and coronary flow reached a maximum level three to five times greater than control. In 45-60 seconds the heart resumed beating; however, coronary flow did not return to control for 5-10 minutes. Hearts were discarded if the peak flow response following occlusion and during the bolus did not reach levels two times and three to five times greater than respective controls. Flow rate following a 250 μg bolus of adenosine was considered maximal for that particular coronary bed. Just prior to termination of an experiment, these same disturbances were again imposed. If the maximal responses produced failed to reach a level 80-90 per cent of that produced by similar measures at the beginning of the experiment the data from the experiment was not included for analysis.

IV. Experimental Protocols

A. Coronary Flow as Affected by Adenosine at Different Levels of Perfusate pH (n = 40)

The following experiments were performed to determine if the coronary vascular response to adenosine is pH sensitive. To assess the effect of pH on adenosine's action, the coronary bed was perfused with a Krebs-Ringer bicarbonate solution equilibrated with 95% O_2-5% CO_2, pH 7.43,

at a constant pressure of 65 cm H_2O achieved by suspending the perfusate reservoir 65 cm above the cannulated heart. When coronary flow and heart rate were stable, a fresh stock solution of adenosine (26.7 mg/100 ml perfusate) (Sigma Grade, Sigma Chemical Co., St. Louis, Mo.) was added to the perfusate reservoir to yield concentrations of adenosine in the perfusate of 10^{-8}, $5x10^{-8}$, 10^{-7}, $5x10^{-7}$, and 10^{-6}M in that order. The dose-effect relationship of adenosine on coronary flow under this condition (perfusate pH 7.43) was compared to subsequent adenosine dose-effect relationships on coronary flow when perfusate pH was something other than 7.43. To determine the new stable flow rate following each addition of adenosine, timed collections of total flow were made in a graduated cylinder. The value obtained from two consecutive collections which were the same was taken as the maximum response to that concentration of adenosine and another volume of stock adenosine solution was added to the reservoir to produce the next highest nucleoside concentration. The maximum flow response to adenosine at each concentration was identified when the stylus tracing on the recorder reached a plateau. When the maximum coronary flow to the greatest concentration of adenosine ($5x10^{-7}$ or 10^{-6}M) was achieved, perfusion of the coronary vasculature was switched to a second, previously prepared test reservoir containing a

perfusate with a different pH. Perfusate in this new reservoir was equilibrated with one of the following test gas mixtures: 1) 98% O_2-2% CO_2, 2) 92% O_2-8% CO_2, 3) 90% O_2-10% CO_2, and 4) 80% O_2-20% CO_2. The coronary vascular bed was perfused from this test reservoir for 10-15 minutes allowing adequate time for a new steady flow rate to occur. Once achieved, the adenosine dose-effect relationship was again examined. This relationship was compared with that produced by the same concentrations of adenosine at pH 7.43. Upon completion of the second dose-effect sequence, the coronary bed was flushed with fresh perfusate equilibrated with 95% O_2-5% CO_2. When flow rate was again stable, the two tests for coronary responsiveness (Section III, B) were again conducted.

In another group of eight hearts the effect of altering the perfusion sequence on adenosine's coronary action was studied. Following stabilization at pH 7.43, hearts were immediately switched to a perfusate of pH 7.20 and an adenosine dose-response relation was studied. Subsequently, hearts were perfused at pH 7.43 and a second adenosine dose-response relation was conducted. Hearts were then perfused with fresh perfusate and tests of coronary responsiveness (Section III, B) were undertaken.

In the above experiments, only two adenosine dose-effect relationships were examined in each heart and the

total time of each experiment was approximately 90-105 minutes. An analysis of variance (RCB design) using factorials was used as an initial test of variance. Means were compared with Tukey's procedure (LSR).

B. Effect of Perfusate pH on Coronary Flow in the Absence and Presence of Adenosine ($8x10^{-7}M$) (n = 7)

In the first part of this series of experiments, only the effect of changing perfusate pH on coronary flow was examined. Hearts were allowed to stabilize while perfusing with solution of pH 7.42. To test the effect of perfusate pH on coronary flow, the PCO_2 of the perfusate was selectively altered by increasing the per cent composition of carbon dioxide in the gas mixture. Perfusate pHs of 7.69, 7.43, 7.20, and 6.89 were used. In a first group of three hearts, the same perfusate sequence was followed. The order was: 7.43 (control pH), 6.89, 7.20 and 7.69. The coronary flow achieved at each pH was recorded. In a second group of four hearts, following perfusion at pH 7.43, a random perfusate pH sequence was utilized. Flow rates in the two groups were compared.

In all cases, perfusate pH was returned to 7.43 and once flow had stabilized, adenosine ($5x10^{-7}M$) was added to the reservoir. pH was again altered. In the first group of three hearts the order was 7.20, 6.89 and 7.69. In the second group of four hearts, the sequence was random.

Total elapsed time for each experiment averaged 50 minutes. Student's t test for paired replicates was used to test if the mean difference ($\bar{d}$) between pairs was greater than zero.

C. Time Course of the Response to Adenosine ($8x10^{-7}$M) at pH 7.20 (n = 5)

This experiment was designed to verify that the attenuation of adenosine coronary dilation by theophylline found in subsequent experiments was due to theophylline and not to a waning with time of the response to adenosine. It also served an additional purpose. If adenosine is to be assigned an important role in regulating coronary blood flow under such conditions as prolonged exercise, it must be shown that adenosine's coronary dilating action can be maintained for an extended period of time. Hearts were allowed to stabilize at pH 7.43. Upon attainment of the steady state for flow, hearts were switched to a perfusate having a pH of 7.20. Ten minutes later, when new steady state conditions for flow were achieved, adenosine solution was added to the reservoir giving a concentration of $8x10^{-7}$M. Coronary flow was continually monitored electromagnetically and flow was measured every 3-5 minutes with a graduated cylinder and stopwatch. Thirty minutes after addition of adenosine to the perfusate hearts were perfused with fresh solution (pH 7.43) and coronary responsiveness (Section III, B) was evaluated. Student's t test for paired replicates

was used to test if the mean difference ($\bar{d}$) between pairs was greater than zero.

D. The Effect of Theophylline on Coronary Flow (n = 12)

To assess the effects of theophylline alone on coronary flow and spontaneous heart rate, hearts were perfused with Krebs-Ringer bicarbonate during which control heart rate and coronary flow were observed. Anhydrous theophylline (1,3-dimethylxanthine, Sigma Chemical Co., St. Louis, Mo.) was dissolved in appropriate volumes of 0.9% saline to produce the stock solutions (10^{-6}-10^{-3}M) used in this study. These stock solutions were then added to the perfusate reservoir to give concentrations of theophylline in the reservoir of 10^{-6}, 10^{-5}, $5x10^{-5}$, 10^{-4}, $5x10^{-4}$ and 10^{-3}M. While perfusing the coronary vascular bed with perfusate containing $5x10^{-5}$M theophylline, aortic inflow was occluded for 30 seconds. The peak flow attained following release of occlusion was compared with that observed in the absence of theophylline. The area under the hyperemic curve and the time required for excess flow to return 50 per cent toward control (T_{50}) were also compared. Following all dose-response tests, the ccronary vascular bed was perfused with fresh perfusate (no theophylline) and coronary responsiveness (Section III, B) was determined. An analysis of variance (RCB design) using factorials was used as an

initial test for variance. Tukey's procedure (LSR) was subsequently used to compare means.

E. Effect of Perfusate pH on Theophylline Attenuation of the Coronary Response to Adenosine ($8x10^{-7}$M) (n = 18)

Should the perfusate pH inhibit theophylline's capacity to attenuate the dilation caused by exogenous adenosine, then one would not expect theophylline to reduce reactive hyperemia since in this state pH is lowered. Thus a series of experiments was performed to test the effect of reducing perfusate pH on the ability of theophylline to attenuate adenosine's coronary dilation. Following stabilization of flow and heart rate, adenosine ($8x10^{-7}$M; chosen because it produced a coronary response similar to but not as great as 10^{-6}M adenosine) was added to the perfusate. Once coronary flow stabilized at a higher rate, theophylline was added to the reservoir to yield concentrations of 10^{-6}, 10^{-5} and 10^{-4}M in that order. Coronary flow was measured at each concentration. The perfusate was then switched to one having a pH of 7.20 (no adenosine or theophylline). Ten to fifteen minutes later when coronary flow was stable, the experiment was repeated. Finally, the vascular bed was perfused with fresh perfusate (pH 7.43, no adenosine or theophylline) and its responsiveness tested (Section III, B).

The pH sequence was reversed in nine hearts. Following stabilization at pH 7.43, a perfusate of pH 7.20 was

introduced. Adenosine and theophylline were added as above. The hearts were then perfused at pH 7.43 and the sequence repeated. Finally the adenosine and theophylline were washed out at pH 7.43 and the responsiveness of the bed was tested. An analysis of variance (RCB design) using factorials was used as an initial test for variance. Tukey's procedure (LSR) was subsequently used to compare means.

F. The Effect on Reactive Dilation, Autoregulation and Hypoxic Dilation of Concurrent Theophylline ($5x10^{-5}M$), Ouabain ($1.4x10^{-7}M$) and Alkalosis (Perfusate pH 7.69) (n = 15)

The potassium and hydrogen ions have also been suggested as mediators of local regulation of the coronary vascular bed. Ouabain can block the vasodilator response to potassium. We therefore decided to test the effect on local regulation of combining theophylline with ouabain and alkalosis.

In a group of eight hearts, following stabilization, inflow was occluded for 30 seconds and upon release, peak coronary flow, total volume of flow for 20 seconds (VCF_{20}), and the time required for excess flow to return 50 per cent toward control (T_{50}) were measured. Approximately five minutes later when coronary flow was stable, the perfusion pressure was quickly increased (by elevating the level of the perfusate reservoir) from 65 cm H_2) to 95 cm H_2O, and the increased rate of flow was recorded. Subsequently the

pressure was dropped to 35 cm H_2O and flow rate at this pressure was also recorded. Following these control maneuvers, hearts were switched to a perfusate containing the above test agents and reactive dilation and autoregulation were again examined. Finally, hearts were perfused with fresh perfusate and coronary responsiveness was determined (Section III, B).

The effect on hypoxic dilation was studied in seven hearts. When coronary flow rate was stable for two consecutive measurements, the perfusate was switched to one equilibrated with 20% O_2-5% CO_2-balance N_2 and the maximum hypoxic flow rate recorded. The perfusate was then replaced with the one equilibrated with 95% O_2-5% CO_2 and flow allowed to return to the prehypoxic control rate. Subsequently, hearts were perfused with solution equilibrated with 20% O_2-2.5% CO_2-balance N_2 and containing theophylline ($5x10^{-5}$M) and ouabain ($1.4x10^{-7}$M). After maximum hypoxic flow rate was recorded, coronaries were perfused with fresh perfusate (equilibrated with 95% O_2-5% CO_2 and containing no test agents) and control flow was again recorded. Hearts were subsequently tested for coronary responsiveness (Section III, B). Student's t test for paired replicates was used to test if the mean difference ($\bar{d}$) between pairs was greater than zero.

RESULTS

I. Coronary Responsiveness to a 250 μg Bolus of Adenosine and Following Release of a 30 Second Inflow Occlusion

Following stabilization of coronary flow and heart rate, the inflow cannula was occluded for 30 seconds then released. Flow increased and then returned to control. Subsequently, a 250 μg bolus of adenosine was rapidly injected into the aortic perfusion tubing slightly proximal to the heart. Eight to twelve cardiac cycles later, the heart stopped in diastole and coronary flow reached a maximum and then returned to control.

Early in these studies it was arbitrarily decided that: 1) the reactive hyperemic flow should be two to three times control flow, 2) the bolus flow should be three to five times control flow, and 3) the postexperimental responses should be greater than 80 per cent of the preexperimental responses to consider the preparation acceptable. Table 1 presents data from nine groups of hearts ($n = 69$) in which pre- and postexperimental data are displayed to illustrate the magnitude and reproducibility of the responsiveness of the coronary vascular bed.

Table 1. Summary of 69 individual experiments (9 series of experiments) showing the magnitude of coronary flow in the isolated guinea pig heart following 30 seconds of inflow occlusion or a 250 µg bolus of adenosine in the pre- and postexperimental states. N = number of replications in each series of experiments. Data are taken from Section IV, Experimental Protocols, and include experiments from parts I, III, IV, and V.

	Reactive Dilation				250 µg Adenosine Bolus			
	Pre-exper.		Postexper.		Pre-exper.		Postexper.	
N	Control	Response	Control	Response	Control	Response	Control	Response
9	6.8±0.6	13.9±0.6	6.9±0.5	13.1±0.8	6.8±0.5	19.0±0.8	6.8±0.5	16.0±0.6
9	5.4±0.3	13.4±0.9	5.7±0.5	12.3±0.9	5.3±0.3	18.6±3.0	5.6±0.4	17.7±2.8
9	5.8±0.24	12.7±0.3	6.0±0.2	12.6±0.4	5.7±0.2	18.4±0.5	6.0±0.2	16.3±0.26
9	6.2±0.41	13.4±0.5	6.3±0.3	12.0±0.6	6.2±0.35	18.7±0.8	6.3±0.3	16.0±0.2
8	6.2±0.25	16.5±0.85	6.0±0.29	15.1±0.92	6.2±0.23	24.1±1.1	6.0±0.31	21.0±1.3
8	5.7±0.33	13.8±0.52	5.7±0.34	13.4±0.61	5.7±0.34	20.4±0.9	5.7±0.36	19.6±0.84
6	5.1±0.29	12.4±0.55	5.3±0.23	12.3±0.67	5.2±0.39	18.8±0.77	5.2±0.29	16.9±1.2
6	5.9±0.39	19.3±0.84	6.1±0.47	17.7±0.66	6.0±0.42	26.8±1.1	6.0±0.46	23.1±0.9
5	6.3±0.22	15.0±0.72	6.1±0.5	14.1±0.66	6.3±0.3	21.9±0.81	6.1±0.42	20.7±0.8

Examination of the table shows that the preparations selected met the above requirements.

II. Coronary Flow Responses to Adenosine (10^{-8}-10^{-6}M) in Perfusates of Different Hydrogen Ion Activities

A. Effect of Lowering the Perfusate pH on the Coronary Flow Response to Adenosine

In experiments conducted on three groups of hearts the perfusate pH was reduced by increasing its PCO_2. The effects of only two perfusate pHs were studied in each experiment. In each group of hearts an adenosine dose-response relation was first established at a perfusate pH of 7.43 $\pm$ 0.01 and this was subsequently compared with an adenosine dose-response relation at a pH other than 7.43 (Table 2, Figures 4, 5 and 6). In the first group of hearts adenosine (10^{-6}M) increased coronary flow to a level which was quantitatively similar at both perfusate pH 7.43 and 7.36. At pH 7.36, control flow and flows at 10^{-8} and 10^{-7}M adenosine were approximately 19 per cent greater than respective flows at pH 7.43. The difference is significant ($P < 0.05$).

In a second group of hearts in which the second dose-response relation was conducted at pH 7.20, the coronary response to 10^{-6}M adenosine was not different than that produced by the same concentration of adenosine at pH 7.43.

Table 2. The effect of lowering the perfusate pH on the capacity of a series of adenosine concentrations to affect flow and heart rate of the isolated guinea pig heart. Hearts in all panels were initially perfused with a perfusate equilibrated with 95% O_2-5% CO_2). Values are expressed as means ± their respective S.E.M., N=9 in panels 1 and 2 and N=6 in panel 3; (*) denotes statistical significance ($P < 0.05$) with respect to pre-experimental values.

		Coronary flow (ml/min)		Heart rate	
	N = 9	Perfusate pH 7.42	7.36	Perfusate pH 7.42	7.36
Adenosine	Pre-exper.	6.2±0.39	7.4±0.26	260±7.0	266±7.1
	10^{-8}M	6.2±0.35	7.5±0.29	260±7.0	265±6.8
	10^{-7}M	7.1±0.37	8.5±0.29	264±7.6	267±5.7
	10^{-6}M	10.9±0.31*	10.9±0.35*	263±7.4	267±6.0
	Postexper.	6.3±0.30		268±5.7	
	N = 9	Perfusate pH 7.42	7.20	Perfusate pH 7.42	7.20
Adenosine	Pre-exper.	5.8±0.24	7.7±0.26	258±10.5	255±8.4
	10^{-8}M	5.7±0.24	8.0±0.35	253±8.9	256±8.7
	10^{-7}M	6.6±0.24	9.9±0.41*	259±8.2	256±8.5
	10^{-6}M	11.3±0.66*	12.1±0.58*	260±8.4	258±8.1
	Postexper.	6.0±0.66		258±8.0	
	N = 6	Perfusate pH 7.43	6.89	Perfusate pH 7.43	6.89
Adenosine	Pre-exper.	5.2±0.16	7.4±0.18	260±2.5	263±5.0
	10^{-8}M	5.2±0.16	7.6±0.20	262±2.0	262±5.7
	$5x10^{-8}$M	5.3±0.22	9.1±0.36	260±2.5	258±4.1
	10^{-7}M	5.9±0.27	10.5±0.32*	262±3.7	258±4.1
	$5x10^{-7}$M	8.9±0.63*	13.7±0.55*	272±2.5*	260±5.1
	Postexper.	4.9±0.15		260±5.1	

Figure 4. The effect of adenosine (10^{-8}-10^{-6}M) on coronary flow in the isolated, spontaneously beating guinea pig heart using two perfusates with different H^+ activities. Hearts were initially perfused with solution of pH 7.42. Subsequently, hearts were switched to a perfusate of pH 7.36 and a second adenosine dose-response relation was studied. *Denotes statistical significance ($P < 0.05$) when compared with respective control (C). N=9 All values are displayed as the mean $\pm$ S.E.M.

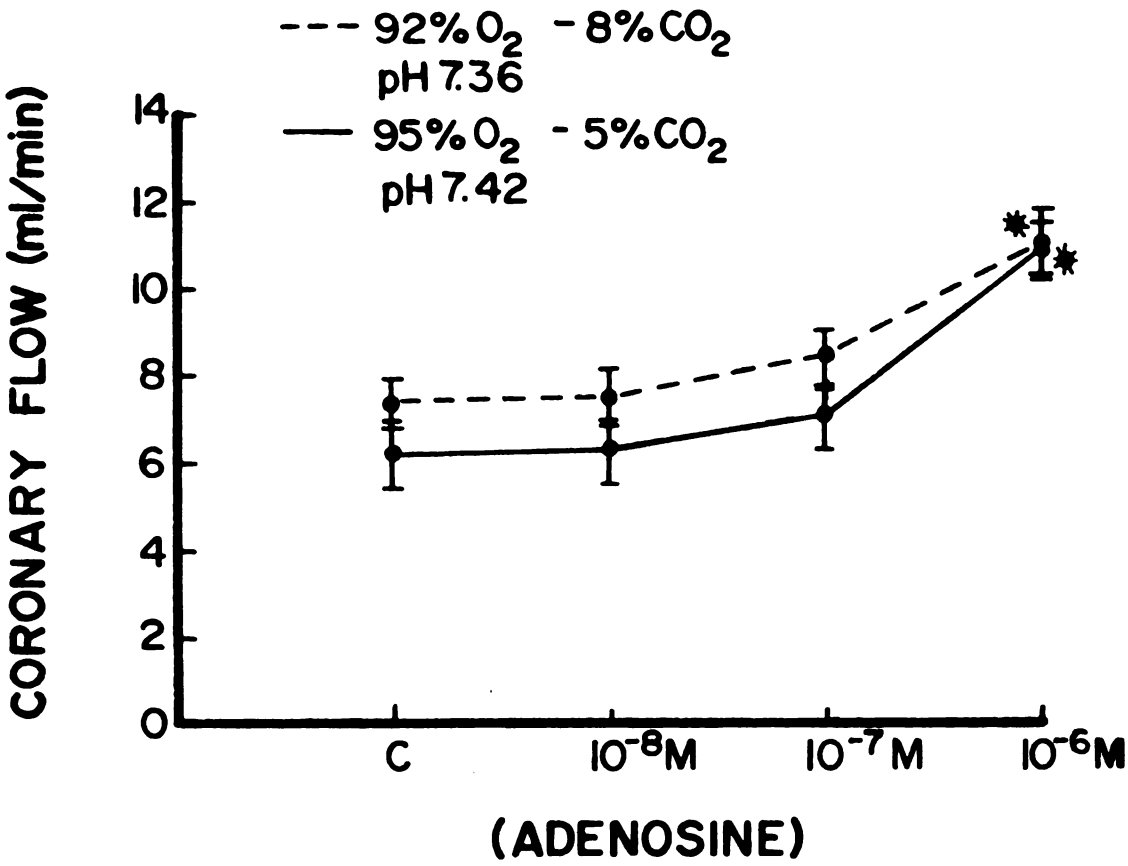

--- 92% O2 - 8% CO2
pH 7.36
— 95% O2 - 5% CO2
pH 7.42
CORONARY FLOW (ml/min)
14
12
10
8
6
4
2
0
C
10-8M
10-7M
10-6M
(ADENOSINE)

Figure 4

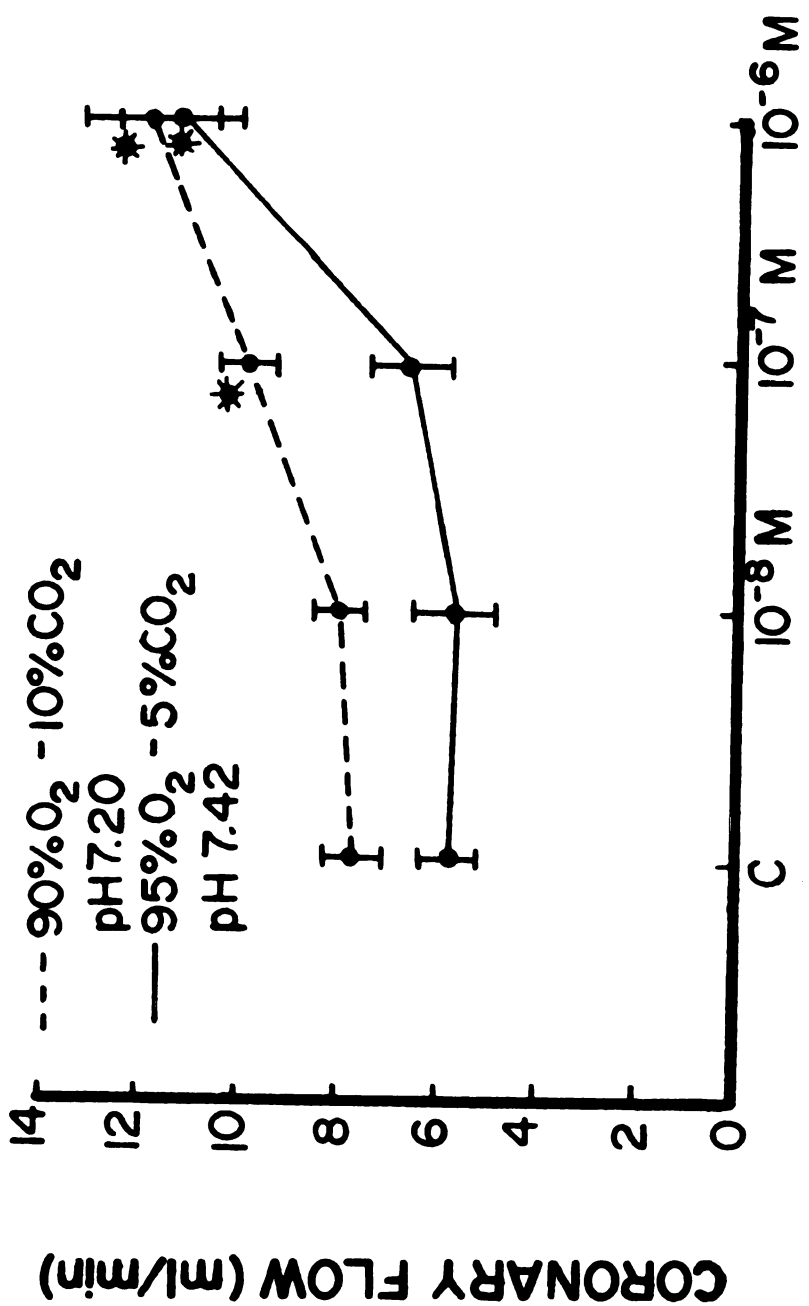


Figure 5: Effect of adenosine on coronary flow in the isolated, spontaneously beating guinea pig heart (hearts perfused first with solution of pH 7.43).

Figure 6. The effect of adenosine (10^{-8}-$5x10^{-7}$M) on coronary flow in the isolated, spontaneously beating guinea pig heart using two perfusates with different H^+ activities. Hearts were initially perfused with solution of pH 7.42. Subsequently, hearts were switched to a perfusate of pH 6.89 and a second adenosine dose-response relation was studied. *Denotes statistical significance ($P < 0.05$) when compared with respective control (C). N=6 All values are displayed as the mean $\pm$ S.E.M.

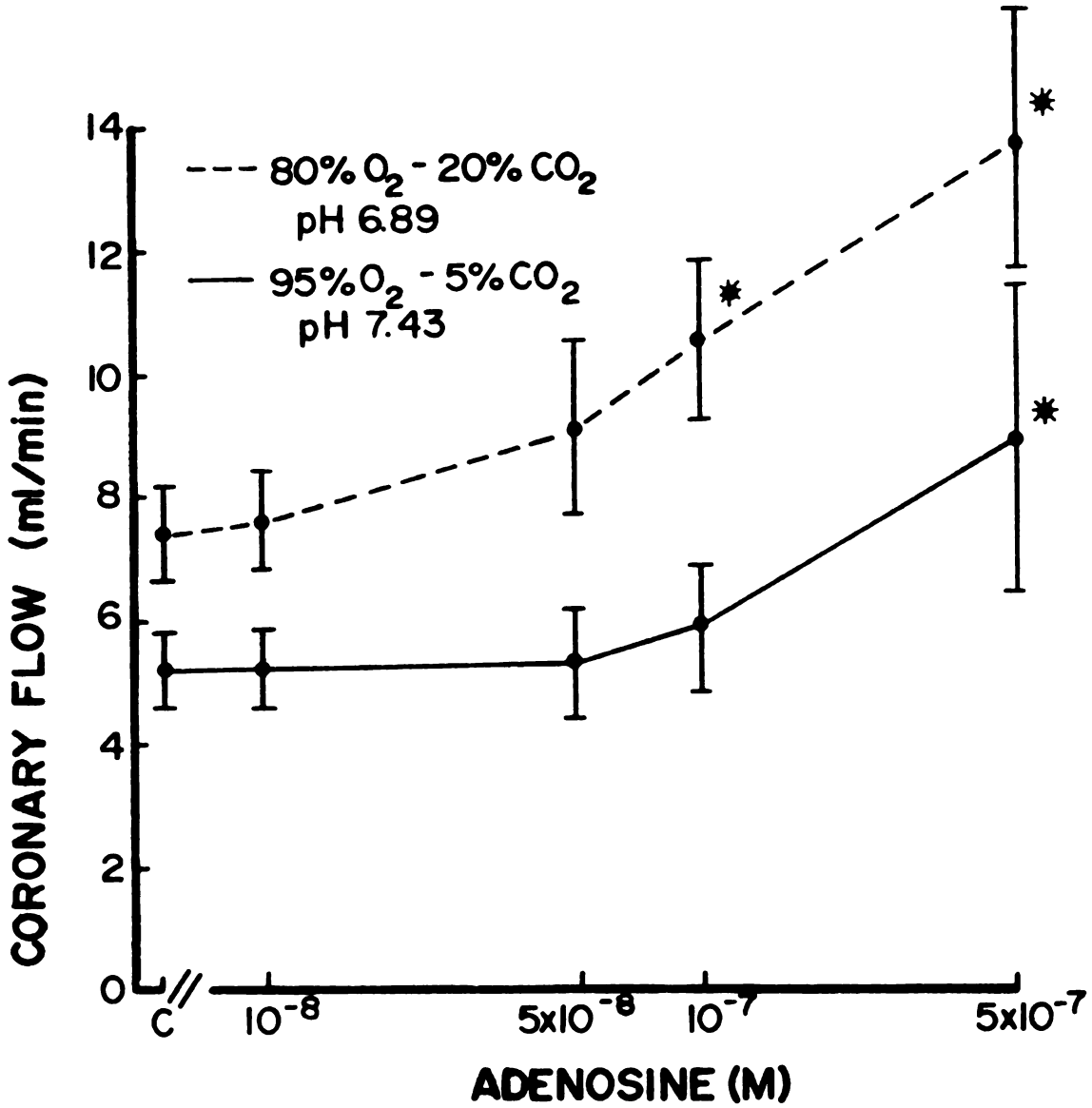

14
12
10
8
6
4
2
0
CORONARY FLOW (ml/min)
--- 80% O2 - 20% CO2
pH 6.89
— 95% O2 - 5% CO2
pH 7.43
*
*
*
C
10-8
5x10-8
10-7
5x10-7
ADENOSINE (M)

Figure 6

However, the initial flow at pH 7.20 (no adenosine) was significantly greater than the control flow at pH 7.43. Further, at pH 7.20, 10^{-7}M adenosine increased flow to a level significantly greater than its respective control. This was not observed at pH 7.43. As in the first group of hearts, flows at 10^{-8} and 10^{-7}M adenosine (both pHs) are different from each other.

In a third group of hearts, the greatest concentration of adenosine used was $5x10^{-7}$M, and the maximum flow achieved at this concentration (Figure 6, bottom curve) was approximately 20 per cent less at pH 7.43 than that produced by 10^{-6}M adenosine in the first two groups of hearts at the same pH. This was not surprising, however, when hearts were subsequently switched to a test perfusate (pH 6.89) adenosine ($5x10^{-7}$M) increased coronary flow to a point noticeably higher than that achieved at either perfusate pH 7.20 or 7.36 in response to 10^{-6}M adenosine (compare bottom curves, Figures 4, 5 and 6). As above, the control flow and those flows produced by each concentration of adenosine at pH 6.89 are different ($P < 0.05$) from respective flows at pH 7.43. The asterisks indicate significant difference from respective controls.

Generally heart rate was not affected by adenosine under any conditions, however, when perfusing hearts with solution of pH 7.43, adenosine ($5x10^{-7}$M) increased heart

rate significantly ($P < 0.05$) from a control of 260 ± 2.5 to 272 ± 2.5 (Table 2, bottom panel).

When statistically analyzing data in Table 2, a randomized complete blocks design (RCB, analysis of variance), using factorials (87), was incorporated to identify an interaction of perfusate hydrogen ion activity and adenosine in producing the observed flow responses. Appendix A displays ANOVA tables with calculated and tabular F values, indicating that when the calculated F value is greater than the tabular F value (in rows headed "interaction") a significant interaction of adenosine and pH has occurred. Note that the calculated F for interaction is nonsignificant at perfusate pH 7.36 (Appendix A, Table A1), but approaches significance at pH 7.20 (Appendix A, Table A2). From these values one could possibly predict a significant F if perfusate pH was reduced below 7.20. Such is the case, for upon further reducing pH to 6.89, a significant ($P < 0.05$) interaction of main effects is revealed (Appendix A, Table A3). Also note in these same three tables that the contribution of perfusate pH to the increase in coronary flow becomes progressively more pronounced as the pH is lowered.

In Figure 7 a regression curve, $b=0.013$, is applied to coronary flow data taken from Figure 5 and was constructed using the method of least squares, where $\hat{y}=\bar{y}+b(x-\bar{x})$.

Figure 7. Regression analysis computed from experiments in which guinea pig hearts were perfused with solution of two H^+ activities. Hearts were exposed to perfusate of pH 7.43 initially and were subsequently perfused with perfusate of pH 7.20. Method of least squares was used in computation, and an analysis of variance determined that the regression curve is linear. b=0.013, N=9, mean $\pm$ S.E.M. are shown on the curve. (● = observed mean flow rates at respective adenosine concentration. x = regression analysis estimates of flow rates.)

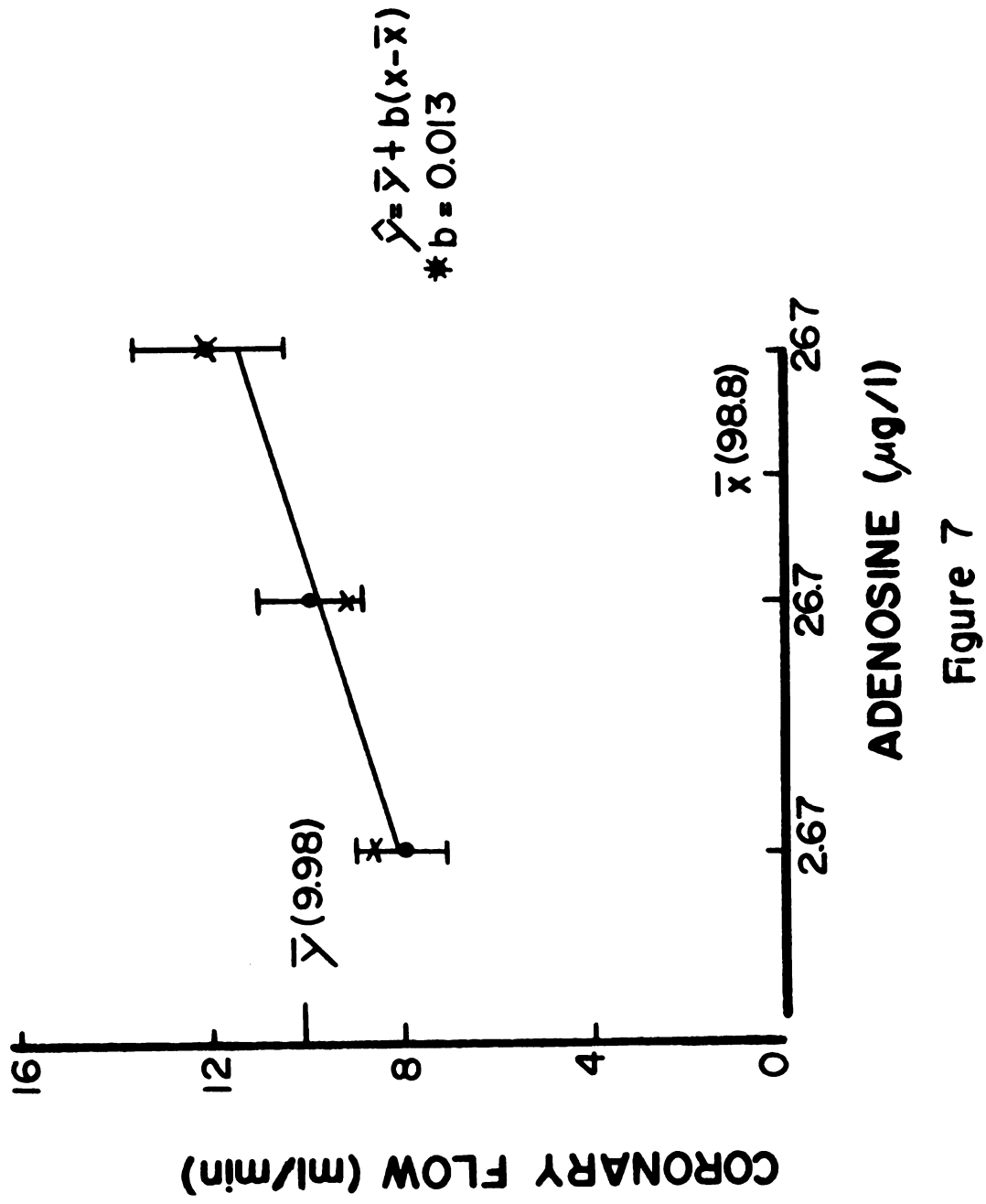

$\hat{y} = \bar{y} + b(x - \bar{x})$
*b = 0.013
$\bar{y}$ (9.98)
$\bar{x}$ (98.8)
CORONARY FLOW (ml/min)
ADENOSINE (μg/l)
16
12
8
4
0
2.67
26.7
267

Figure 7

An analysis of variance revealed significant linearity of the curve. The regression curve illustrates (when the curve is linear) that if one were to use adenosine concentrations greater than 2.67 µg/l but less than 267 µg/l, flow would increase as adenosine concentration increased and that the pattern of the response would be similar to that observed in Figure 7.

B. The Effect of First Perfusing the Coronary Vessels with Perfusate of pH 7.20 on Adenosine's Dilating Action

Early in the adenosine studies, the question was posed: Does one see the same coronary flow response to adenosine when hearts are perfused initially with solution of a pH other than 7.42 then subsequently perfused with solution of pH 7.42? To investigate this question coronary vasculature was exposed to control perfusate (pH 7.42) during the stabilization period. Upon reaching the steady state flow, hearts were immediately switched to a reservoir with perfusate of pH 7.20. The previously established stabilization flow (pH 7.42) was regularly increased by approximately 25 per cent (5.7±0.33 to 7.1±0.5 ml/min) by perfusate of pH 7.20. Altering the order in which hearts were perfused had a marked effect on the coronary action of adenosine. Figure 8 shows that the flow response to adenosine (10^{-6}M) at pH 7.42 was similar to responses seen in our previous experiments using the same concentration

of adenosine and the same perfusate pH (Figures 4 and 5). However, the response to adenosine (10^{-6}M) at pH 7.20 (Figure 8) was approximately 30 per cent greater than that produced when hearts were perfused secondly at pH 7.20 (Figure 5), and 34 per cent greater than that at pH 7.42 in the present study. Note that 10^{-7}M adenosine (pH 7.42) had no effect on coronary flow whereas the same concentration of adenosine at pH 7.20 increased mean flow markedly above its respective control. An interesting finding is that the flow responses to adenosine ($5x10^{-7}$ and 10^{-6}M) at pH 7.20 are not significantly different from each other and flow appears to be reaching a plateau at 10^{-6}M. Adenosine ($5x10^{-7}$ and 10^{-6}M) responses at pH 7.42 are significantly ($P < 0.05$) different from each other and flow gives no signs of reaching a plateau at $5x10^{-7}$M adenosine.

To facilitate inspection of adenosine's coronary action at pH 7.20 as affected by varying the pH perfusion order, the top curves from Figures 5 and 8 were used to construct Figure 9. Inspection of control flow rates (7.7 and 7.1 ml/min) in each curve in Figure 9 suggests that the difference in the flow responses to 10^{-6}M adenosine cannot be accounted for by the magnitude of difference (0.6 ml/min) in respective control flows, but rather must be related to an effect of the order of perfusion on adenosine's dilating capacity.

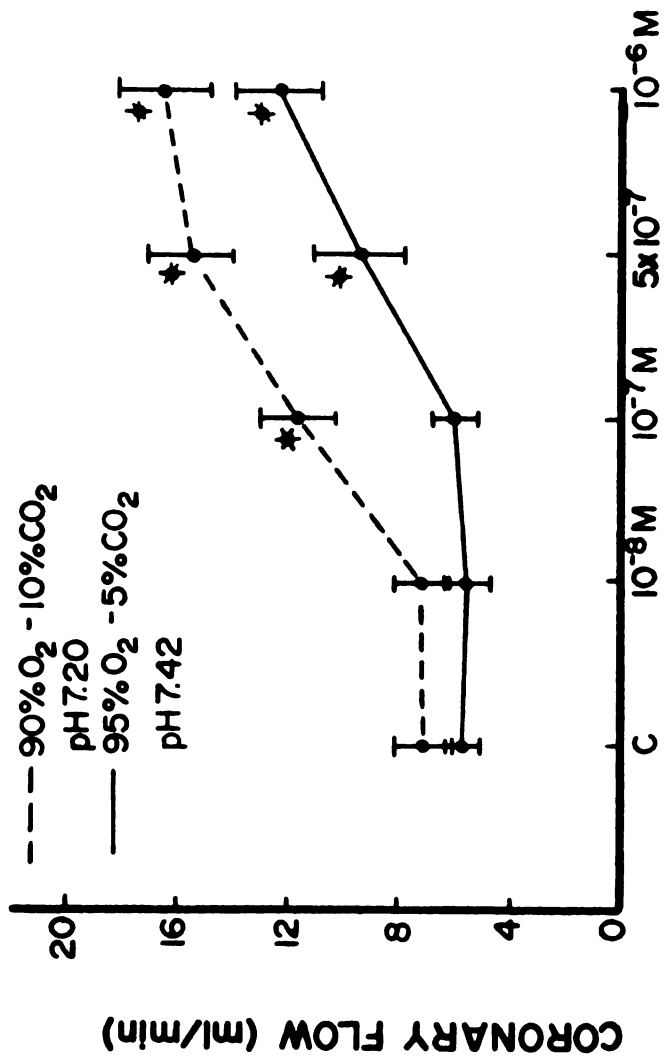


Figure 8: Effect of adenosine on coronary flow in the isolated, spontaneously beating guinea pig heart (hearts perfused first with solution of pH 7.20). C = control. (*) indicates statistical significance when compared with control. N=8

Figure 9. This figure illustrates data taken from Figures 5 and 6. The solid curve shows the effects of adenosine on coronary flow at a perfusate of pH 7.20 in hearts first perfused at pH 7.42. The broken curve presents adenosine's coronary action when hearts were perfused first at pH 7.20 followed by perfusion at pH 7.42. The points on each curve are the mean $\pm$ S.E.M. Mean heart weight (----) = 1.2 gm, mean heart weight (——) = 1.1 gm. N = 18

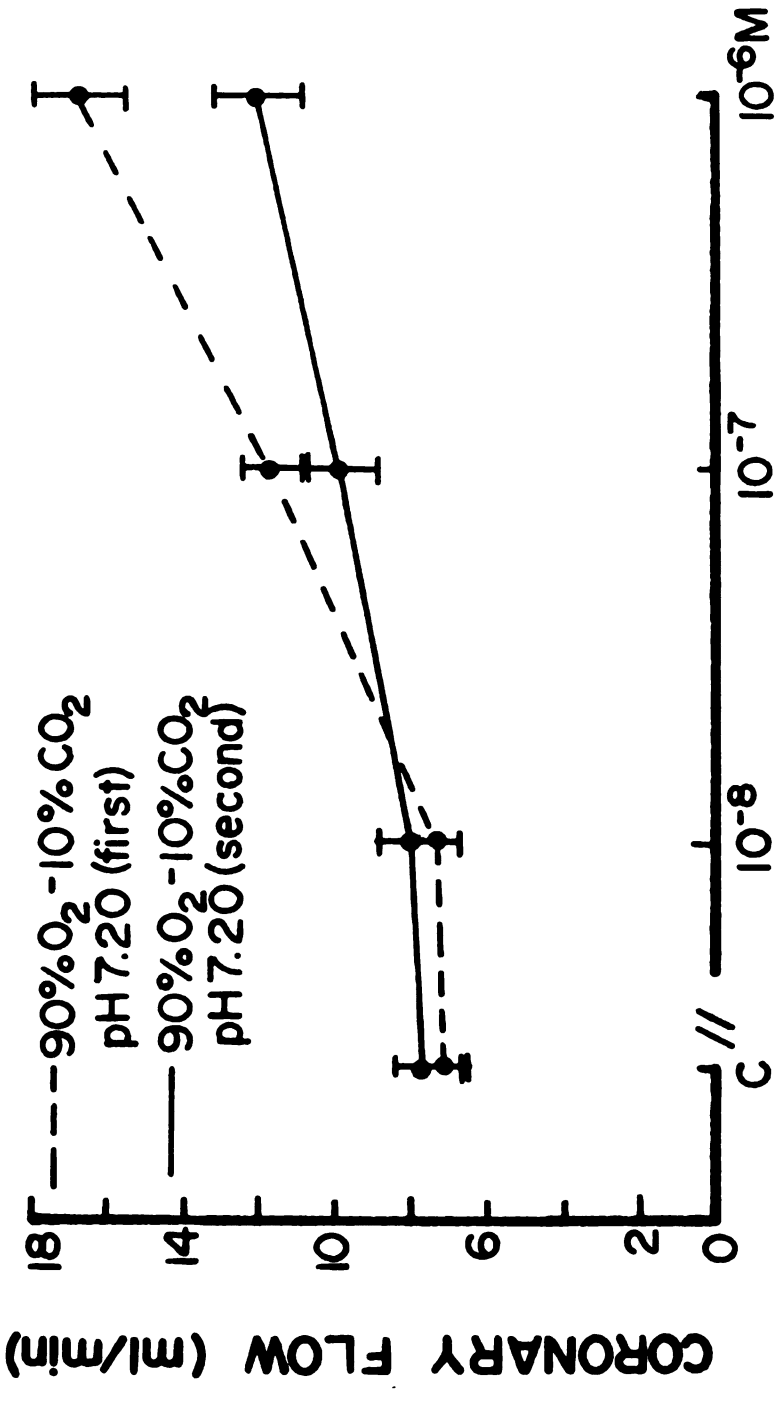

CORONARY FLOW (ml/min)
18
14
10
6
2
0
C
10-8
10-7
10-6M
ADENOSINE(M)
90%O2-10%CO2
pH 7.20 (first)
90%O2-10%CO2
pH 7.20 (second)

Figure 9

Mean heart weight in the two groups was 1.2 $\pm$0.005 and 1.1 $\pm$ 0.06 gm respectively.

Table 3 indicates that at both perfusate pH 7.20 and pH 7.43, adenosine (10^{-6}M) increased heart rate significantly ($P < 0.05$). However, Table 2 shows that this same concentration of adenosine at pH 7.42 and pH 7.20 had no effect on heart rate when the perfusate sequence was not altered. Thus the increase in flow at pH 7.20 in the former (Table 3) might be partially attributable to the increased heart rate.

C. The Effect of Increasing Perfusate pH on the Coronary Flow Response to Adenosine (10^{-8}-$5x10^{-7}$M)

The results of experiments in which adenosine's action on the coronary vessels was investigated in the presence of perfusates of increased hydrogen ion activities (Table 2, Figures 4, 5 and 6) indicated that adenosine not only retained its capacity to dilate when the pH of perfusing fluid was lowered, but that its coronary actions were enhanced by an interaction with hydrogen ion. Our next approach was the obvious; to investigate the effect on adenosine coronary dilation of increasing the pH of the perfusate above 7.42. This was accomplished equilibrating perfusate with 98% O_2-2% CO_2, yielding a perfusate pH of 7.69. Hearts were first perfused with solution of pH 7.42, and adenosine ($5x10^{-7}$M), increased mean coronary flow from

Table 3. Coronary flow and heart rate in response to adenosine in perfusates of two different $[H^+]$. Hearts were first subjected to pH 7.20 followed by pH 7.43. All values are expressed as means ± S.E.M. (*) denotes statistical significance ($P < 0.05$). N = 8)

		Coronary flow (ml/min		Heart rate	
		pH 7.20	pH 7.43	pH 7.20	pH 7.43
Adenosine	Pre-exper.	7.1±0.5	5.7±0.33	248±5.1	254±4.2
	10^{-8}M	7.2±0.5	5.5±0.38	248±5.1	254±4.2
	10^{-7}M	11.7±0.67*	6.0±0.40	252±6.0	257±5.5
	$5x10^{-7}$M	15.6±0.77*	9.5±0.80*	255±6.7	257±4.5
	10^{-6}M	16.6±0.83*	12.4±0.80*	263±7.3*	269±6.0*
	Postexper.		5.7±0.34		256±5.0

6.2 ± 0.24 ml/min to 12.8 ± 0.8 ml/min, an increase of 106 per cent (Table 4, Figure 10). When hearts were subsequently perfused with solution at pH 7.69, control flow fell insignificantly to 5.8 ± 0.29 ml/min, and this was increased only to 8.6 ± 0.57 ml/min in the presence of $5x10^{-7}$M adenosine (Figure 10). This represents a change of only 2.8 ml/min above control as compared to a change of 6.6 ml/min at pH 7.42 in response to the same concentration of adenosine. Examination of Table 5 reveals that similar results were produced when diastolic and systolic flow rates were compared to their respective controls at both levels of perfusate pH. The only statistically significant ($P < 0.05$) increases in flow at pH 7.69 occurred in response to $5x10^{-7}$M adenosine. Heart rate was not affected by adenosine ($5x10^{-7}$M) at perfusate of pH 7.69.

III. Coronary Flow Response to Adenosine ($8x10^{-7}$M) at Perfusate pH 7.20 for 30 Minutes

This experiment was designed to verify the findings of subsequent experiments in which theophylline was used to attenuate coronary dilation produced by adenosine. If the adenosine response does not want with time, then it is unlikely that attenuation by theophylline would be partially due to waning of the adenosine response. Further, since the flow responses to $5x10^{-7}$ and 10^{-6}M adenosine in hearts

Table 4. Adenosine's effects on mean, diastolic and systolic coronary flow (ml/min) and on spontaneous heart rate. Hearts were initially perfused with solution of pH 7.42 followed by subsequent perfusion at pH 7.69. (*) denotes statistical significance ($P < 0.05$) with respect to pre-experimental value. All flow values are the mean while heart values are the mean $\pm$ S.E.M. N = 8

Adenosine	pH of Perfusing Solution							
	Mean		Diastolic		Systolic		Heart Rate	
	7.42	7.69	7.42	7.69	7.42	7.69	7.42	7.69
Pre-exper.	6.2	5.8	10.6	9.6	2.6	2.2	251±4.8	254±4.8
10^{-8}M	6.1	5.8	10.4	9.6	2.7	2.2	251±4.8	254±4.8
$5x10^{-8}$M	6.7	6.2	11.3	10.1	2.8	2.1	248±4.5	252±4.5
10^{-7}M	7.8	6.3	12.4	10.1	3.3	2.1	249±5.4	255±4.4
$5x10^{-7}$M	12.8*	8.6*	20.3*	13.5*	6.9*	4.2*	258±4.5	262±4.8
Postexper.	6.0		10.5		2.4		254±4.8	

Figure 10. This figure compares the effects on coronary flow (ml/min) of adenosine (10^{-8}-$5x10^{-7}$M) at perfusate pH 7.42 and 7.69. The top two curves depict diastolic flow, the middle two depict mean coronary flow, and the two lower curves represent systolic flow. All points are presented as means + respective S.E.M. (*) statistically significant ($P < 0.05$) when compared to control (C). N = 8

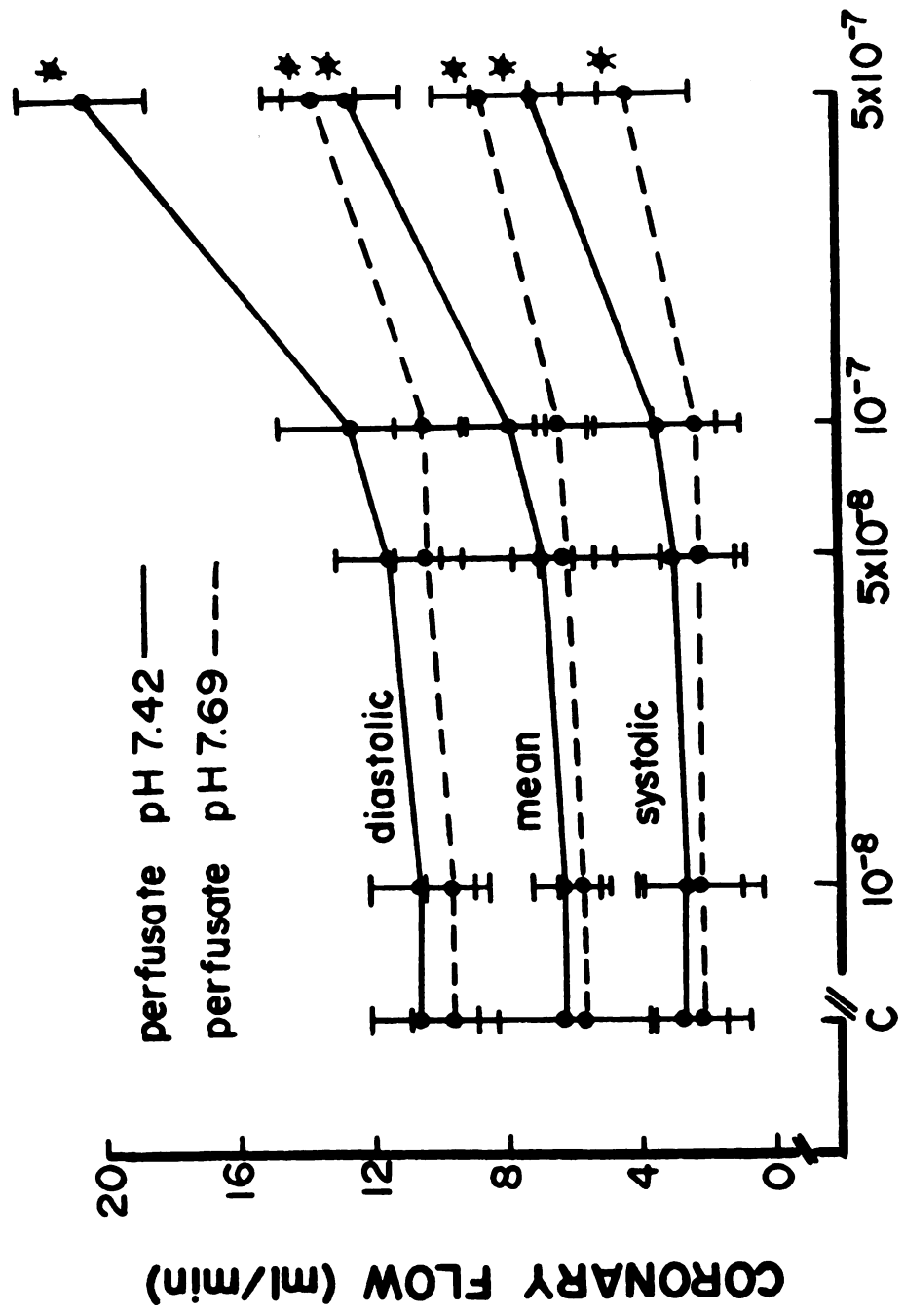

CORONARY FLOW (ml/min)
20
16
12
8
4
0
perfusate pH 7.42
perfusate pH 7.69
diastolic
mean
systolic
C
10⁻⁸
5x10⁻⁸
10⁻⁷
5x10⁻⁷
ADENOSINE (M)

Figure 10

in which the perfusate sequence was reversed (perfused at pH 7.20 immediately after stabilization at pH 7.42) appeared to be plateauing, it was of interest to see if the coronary vasculature would remain dilated for an extended period of time. To study this possibility, hearts were perfused with solution of pH 7.20 and adenosine ($8x10^{-7}M$) was added to the perfusate. Coronary flow was monitored for approximately 30 minutes and flow was collected each 3-5 minutes with a graduated cylinder. Flow regularly reached a statistical maximum 5 minutes after addition of adenosine to the perfusate (flow at this time was not significantly different from that measured at 10 minutes; Figures 11 and 12), and was found to have increased two and a half times above control. Twenty-five minutes later (30 minutes following adenosine administration) coronary flow was still two and a half times greater than control. Table 5 shows that coronary flow in these experiments was calculated and expressed as both the per cent increase above control flow, and as the per cent of maximum flow. As can be seen (Table 5, Figure 12) 30 minutes following addition of adenosine, flow was still equal to 99 per cent of the response seen at minute 5. Figure 11 is a representative tracing taken from one experiment.

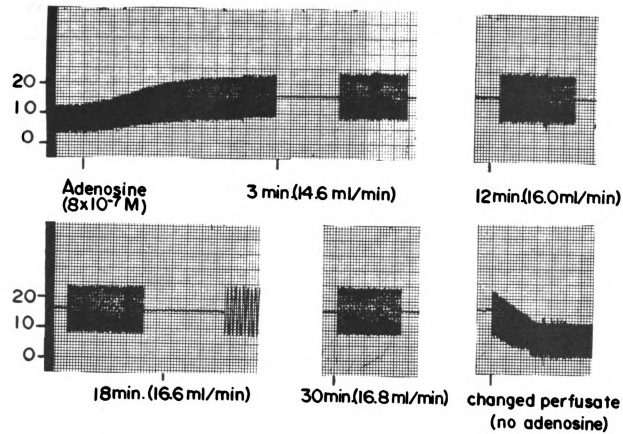


Figure 11: Adenosine (8×10^{-7} M) on coronary flow for 30 min. at perfusate pH 7.20.

Table 5. Effect of adenosine on coronary flow and heart rate over a 30 min. period during which the heart was perfused with a solution of pH 7.20. Coronary flow produced by adenosine reached a maximum at 5 min., consequently all subsequent flow rates were statistically ($P < 0.05$) compared to this value and re-expressed both as the per cent above control and as the per cent of maximum flow. N = 9

	Time (min.) Following the Administration of Adenosine (8×10^{-7} M)							
	0	3	5	10	15	20	25	30
Flow (ml/min)	6.3±0.41	8.6±0.5	16.4±0.9	16.9±0.9	16.7±1.1	16.5±1.2	16.3±1.4	16.3±1.2
% above control		37	160	168	165	162	159	159
% of max. flow		52	100	103	102	101	99	99
Heart rate	233±8.9	233±8.7	236±6.4	236±6.4	237±7.9	238±8.7	238±8.7	238±8.5

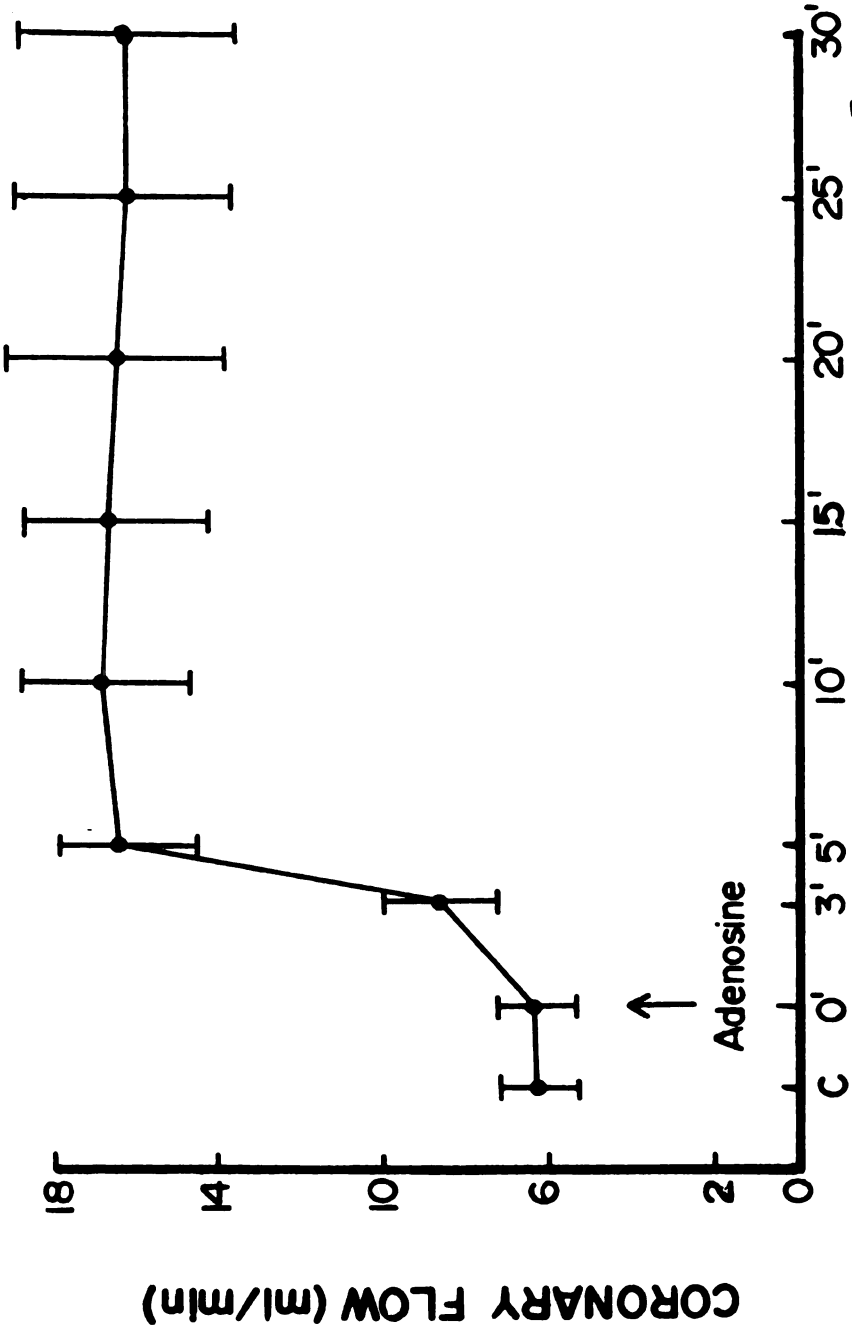


Figure 12: Coronary flow as affected by adenosine (8×10^{-7} M) over a period of 30min. at perfusate pH 7.20. N=5

IV. The Effects on Coronary Flow of Changing the pH of the Perfusate in the Absence and Presence of a Single Concentration of Adenosine ($5x10^{-7}M$)

In this series of experiments we examined the effect of changing perfusate pH, in the absence and presence of adenosine, on coronary flow. To alter the pH, perfusate was selectively equilibrated with four experimental gas mixtures: 1) 95% O_2-5% CO_2, 2) 98% O_2-2% CO_2, 3) 90% O_2-10% CO_2, and 4) 80% O_2-20% CO_2. To determine if progressively lowering or raising the pH of the perfusate produced an additive or inhibitory action on the observed coronary flow response, the first three hearts in a group of seven hearts were exposed to the same sequence of perfusion: hearts were initially perfused at pH 7.43, followed by 6.89, 7.20 and 7.69 (Table 6, middle panel). A subsequent group of four hearts was perfused randomly following perfusion at pH 7.43 (Table 6, bottom panel). Note that the results are similar in both groups; coronary flow progressively increased as perfusate pH was lowered. The top panel in Table 6 presents means $\pm$ S.E.M. from grouping the results in the other two panels and emphasizes the fact that regardless of the order of perfusing solution at different levels of pH, the results are similar. That coronary flow in the absence of adenosine is linearly regressed on perfusate pH is illustrated in Figure 14 which is a negative

Table 6. Effect of perfusates of differing pH (6.89-7.69) on coronary flow (ml/min) and heart rate in the absence and presence of adenosine ($5x10^{-7}M$). The top panel presents mean values ± S.E.M. from 7 hearts. Of these hearts, three were perfused in the same order: perfusate pH 7.42, 7.69, 7.20, 6.89. Data from these three appear separately in the middle panel. The bottom panel shows data from the other four which were perfused randomly following perfusion at pH 7.42. In each case, the response to adenosine was compared to its respective control (*), and to the adenosine response at pH 7.43 (#). Control flow rates were compared to the flow rate at pH 7.43 (●). $P < 0.05$

pH	Control	Adenosine	Heart Rate
7.69±0.06	5.3±0.35	6.9±0.48*#	257±6.85
7.43±0.01	6.3±0.32	8.6±0.38*	255±7.37
7.20±0.02	7.2±0.27●	13.0±0.60*#	257±7.00
6.89±0.06	9.1±0.50●	18.6±1.1*#	255±8.9
7.68±0.08	4.8±0.7●	6.1±0.81*#	256±16.02
7.45±0.06	6.4±0.64	7.7±0.39*	255±16.4
7.18±0.07	7.3±0.47●	12.0±0.87*#	259±14.9
6.87±0.02	9.4±0.46●	16.3±1.43*#	256±21.2
7.70±0.04	5.6±0.3	7.6±0.41*#	258±6.0
7.41±0.06	6.3±0.39	9.2±0.29*	255±7.5
7.21±0.04	7.2±0.38●	13.8±0.53*#	255±7.5
6.90±0.33	8.9±0.85●	20.2±1.05*#	255±7.5

regression curve (b=-4.80) constructed by the method of least squares $\hat{y}=\bar{y}+b(x-\bar{x})$ and tested for significance of linearity by an analysis of variance (Appendix A, Table A7). A 95% confidence interval ($b \pm {}^{t}0.05(5)\ 0.62$) accompanies the regression curve.

Figure 13 and Table 6 illustrate that the apparent enhanced ability of adenosine to dilate the coronaries as the perfusate pH is lowered cannot be accounted for by adding to adenosine's coronary effect the increase in flow due to pH alone.

An interesting comparison was made between the coronary flow response produced by the 250 µg bolus of adenosine used routinely at the beginning of all experiments and the adenosine (5×10^{-7}M) response at pH 6.89 in the current experiments. Bunger et al. (11) reported that the decrease in coronary resistance in a similar guinea pig heart preparation in response to 250 µg adenosine appeared to be maximal. In this series of experiments we found that at pH 6.89 adenosine (5×10^{-7}M) produced a flow response quantitatively similar to that produced by a 250 µg bolus (compare top panel, Table 6 with Table 1, column 6).

Spontaneous heart rate was not affected by the pH of the perfusate or by adenosine (Table 6).

Figure 13. The effect of perfusate pH on coronary flow in the absence and presence of 5×10^{-7} M adenosine. All hearts were initially perfused at pH 7.43, and the coronary response to adenosine was first examined at pH 7.43. Data are displayed as mean values + S.E.M. N = 7

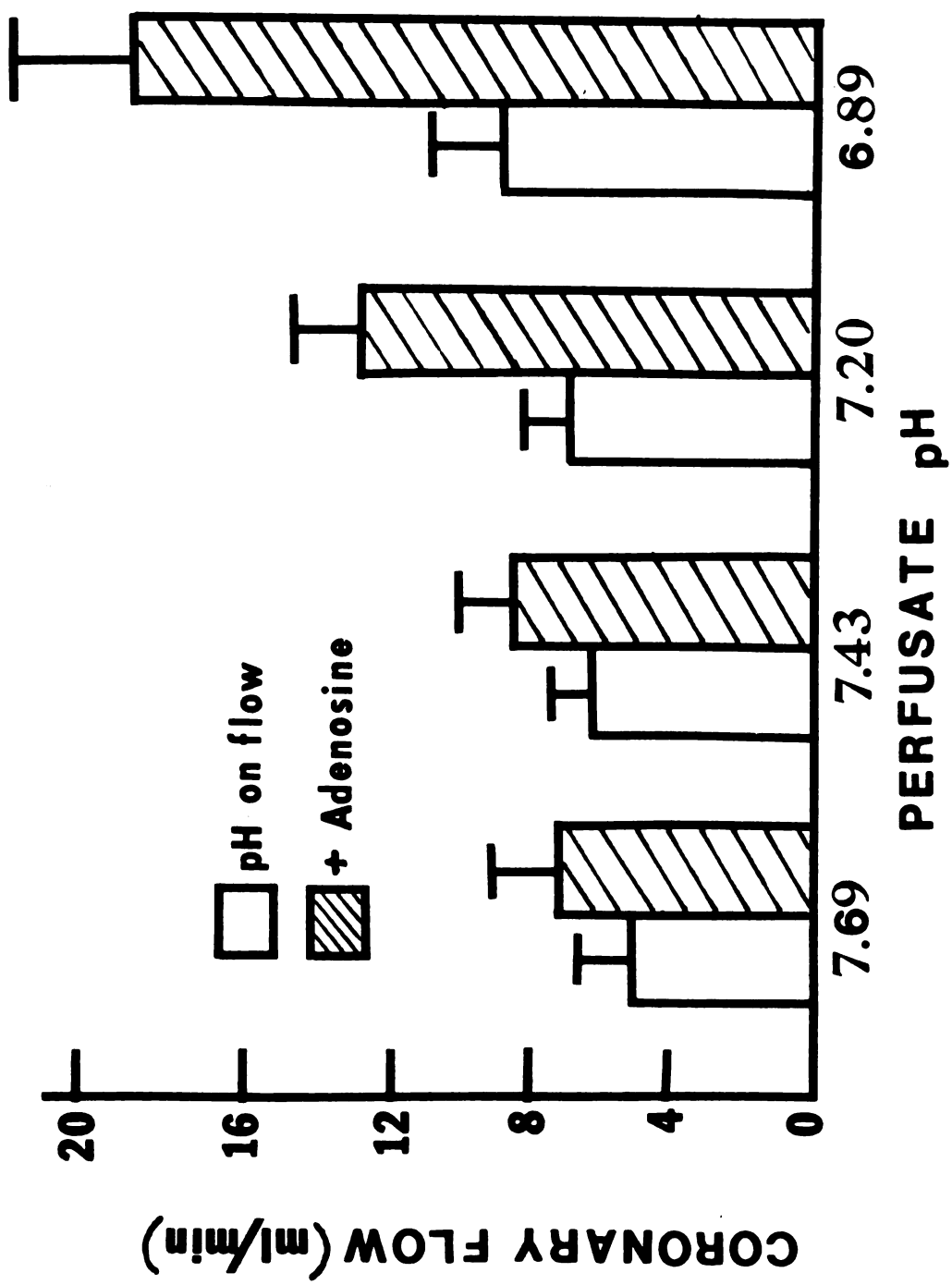

CORONARY FLOW (ml/min)
20
16
12
8
4
0
pH on flow
+ Adenosine
7.69
7.43
7.20
6.89
PERFUSATE pH

FIGURE 13

Figure 14. Linear regression of coronary flow on perfusate pH; method of least squares used for computation, linearity tested with an analysis of variance. N = 7 (● = observed mean values for coronary flow at respective pHs.)

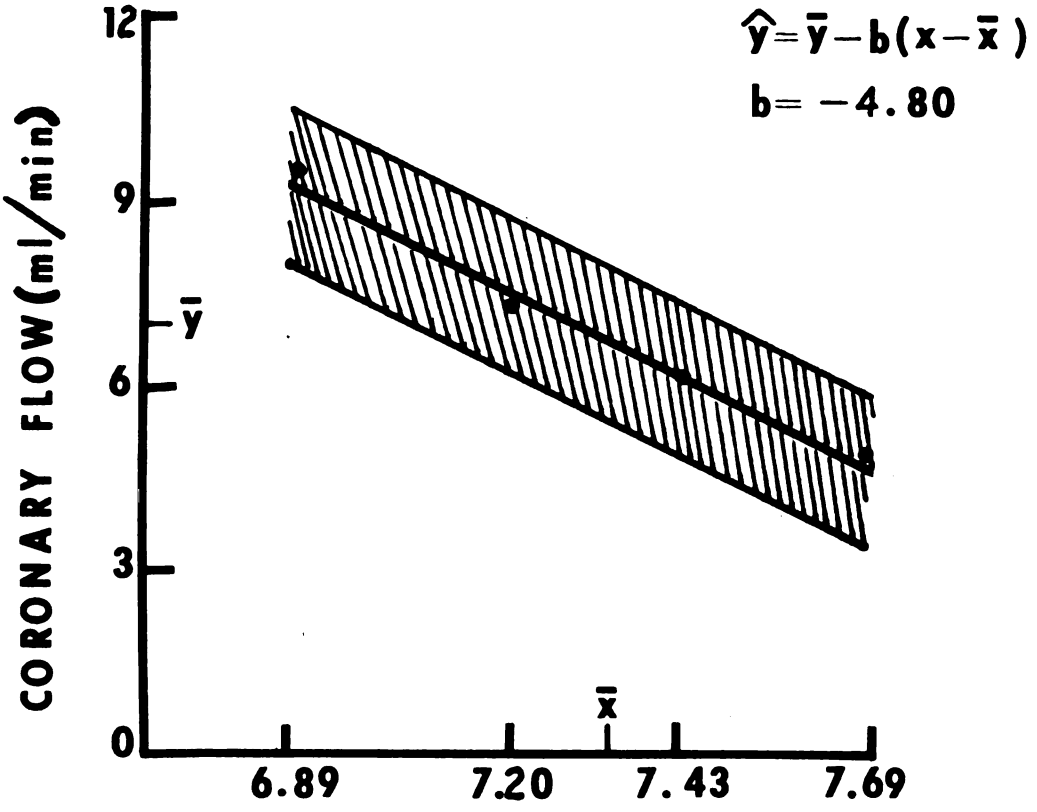

$\hat{y}=\bar{y}-b(x-\bar{x})$
b= −4.80
12
9
6
3
0
$\bar{y}$
$\bar{x}$
6.89
7.20
7.43
7.69
CORONARY FLOW (ml/min)
PERFUSATE pH

FIGURE 14

V. The Effects of Theophylline (10^{-6}-10^{-3}M) on Coronary Flow and Heart Rate

Reports in the literature are controversial with respect to the effects of theophylline on reactive hyperemia, and on the increased coronary blood flow produced in response to hypoxia. The nonuniformity of these reports has cast doubt on the hypothesis that adenosine is the metabolic mediator of coronary blood flow. In the following experiments the objective was to see if the capacity of theophylline to attenuate adenosine coronary dilation was altered by changing the hydrogen ion activity of the perfusate.

Table 7 and Figure 15 present results from preliminary experiments in which we studied the direct action of theophylline on coronary flow and heart rate to find concentrations which could be used to attenuate adenosine's coronary action but which by themselves were without a measurable effect on the heart. Concentrations of theophylline ranging from 10^{-6} to 10^{-4}M were investigated at two levels of perfusate pH (7.43 and 7.20), while $5x10^{-4}$ and 10^{-3}M theophylline were later investigated in a perfusate of pH 7.43 (Figure 17). Generally, coronary flow was not affected by theophylline (10^{-6}-10^{-4}M); however, when hearts were exposed to $5x10^{-4}$ or 10^{-3}M theophylline cororary flow was significantly ($P < 0.05$) increased to 7.3 ± 0.61 and

Table 7. Effect of theophylline (M) on steady-state coronary flow in two groups of hearts (top and middle panels). Bottom panel shows coronary flow following a 30 sec. occlusion of inflow in the presence and absence of theophylline. Data are presented as means ± S.E.M. (*) denotes statistical significance ($P < 0.05$) when compared to respective pre-experimental values.

	Coronary Flow (ml/min/gm)		Heart Rate	
N = 6	95% O_2-25% CO_2	90% O_2-10% CO_2	95% O_2-5% CO_2	90% O_2-10% CO_2
Pre-exper.	5.0±0.26	5.6±0.32	249±8.2	248±11.7
10^{-6}	4.9±0.25	5.6±0.26	243±7.6	252±10.0
10^{-5}	4.7±0.23*	5.5±0.26	248±7.4	252±10.0
10^{-4}	5.0±0.22	5.9±0.26*	254±8.4	254±9.7
Postexper.	5.1±0.29		256±8.6	

	Coronary Flow (ml/min)	Heart Rate
N = 5	95% O_2-5% CO_2	95% O_2-5% CO_2
Pre-exper.	6.1±0.49	264±13.5
$5x10^{-4}$	7.3±).61*	289±17.0*
10^{-3}	10.3±0.66*	334±10.5*
Postexper.	6.0±0.46	279±9.3*

	Effect pf Theophylline ($5x10^{-5}$M) on Reactive Dilation		
N = 6	Pre-exper.	Exper. ($5x10^{-5}$M)	Postexper.
Peak flow (ml/min)	12.4±0.55	12.2±0.67	12.1±0.66
Duration (T_{50})	17.5 sec.	18.5 sec.	21.0 sec.
Area under curve (sq. cm.)	5.6	4.9	5.5

Figure 15. Effect of theophylline (M) on coronary flow and spontaneous heart rate at two levels of perfusate pH. Hearts were perfused initially with solution of pH 7.42 and were subsequently perfused with solution of pH 7.20.

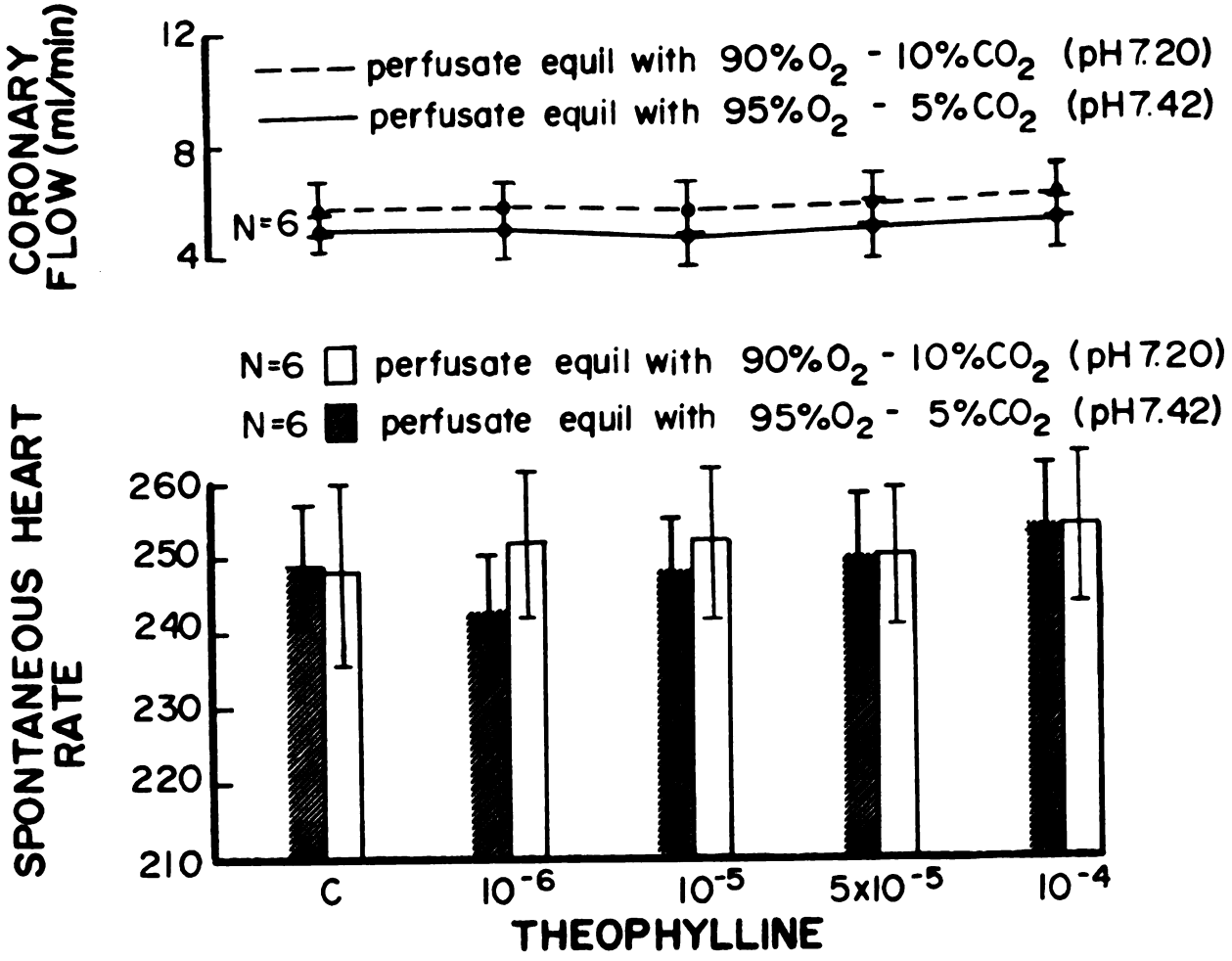

CORONARY FLOW (ml/min)
12
8
4
N=6
perfusate equil with 90%O2 - 10%CO2 (pH 7.20)
perfusate equil with 95%O2 - 5%CO2 (pH 7.42)
SPONTANEOUS HEART RATE
N=6 perfusate equil with 90%O2 - 10%CO2 (pH 7.20)
N=6 perfusate equil with 95%O2 - 5%CO2 (pH 7.42)
260
250
240
230
220
210
C
10-6
10-5
5x10-5
10-4
THEOPHYLLINE

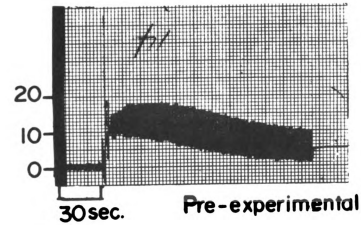
Figure 15

10.3±0.66 ml/min (Figure 17, Table 8). Subsequent perfusion with fresh solution containing no theophylline quickly returned flow to its pre-experimental state.

While perfusing hearts in the presence of $5x10^{-5}$M theophylline, the coronary response to an inflow occlusion of 30 seconds was observed and the peak rate of coronary flow was compared with pre- and postexperimental responses to occlusion observed in the absence of theophylline (Table 7, bottom panel). Figure 16 shows representative responses to inflow occlusion in the absence (pre- and postexperimental) and in the presence of theophylline and shows no significant difference in peak flow as measured. Although the shapes of the curves do appear to be different subsequent comparison indicated that the time for excess flow to return half way to control was not significantly affected by theophylline. In quantitating the area under the diastolic portion of the curve by planimetry, it was found that theophylline decreased this area by only 8 per cent.

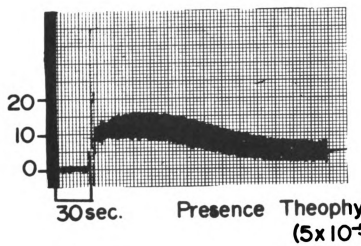
Heart rate was unaffected by 10^{-6}-10^{-4}M theophylline at either pH 7.43 or pH 7.20 (Figure 15). When the second group of hearts was subsequently exposed to $5x10^{-4}$ and 10^{-3}M theophylline, heart rate was significantly ($P < 0.05$) increased from a pre-experimental value of 264±13.5 to 289±17.0 and 334±10.5 beats/min respectively (Figure 17). Table 8 shows that after 10-15 minutes of perfusing the

Figure 16. The effect of theophylline ($5x10^{-5}M$) on reactive dilation. The top and bottom panels show typical responses in the pre- and post-experimental states. During perfusion of hearts with solution of pH 7.42 (middle panel) theophylline was added to the reservoir prior to occluding inflow. N = 6

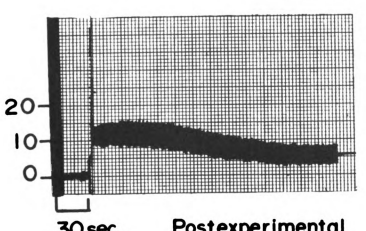


Peak Flow

13.0ml/min.



12.0ml/min.



12.0ml/min.

Figure 16

Figure 17. Effect of theophylline on coronary flow and spontaneous heart rate when hearts were perfused with solution of pH 7.43. Data are presented as means ± S.E.M. (*) denotes statistical significance ($P < 0.05$) when compared with respective control (C). N = 5

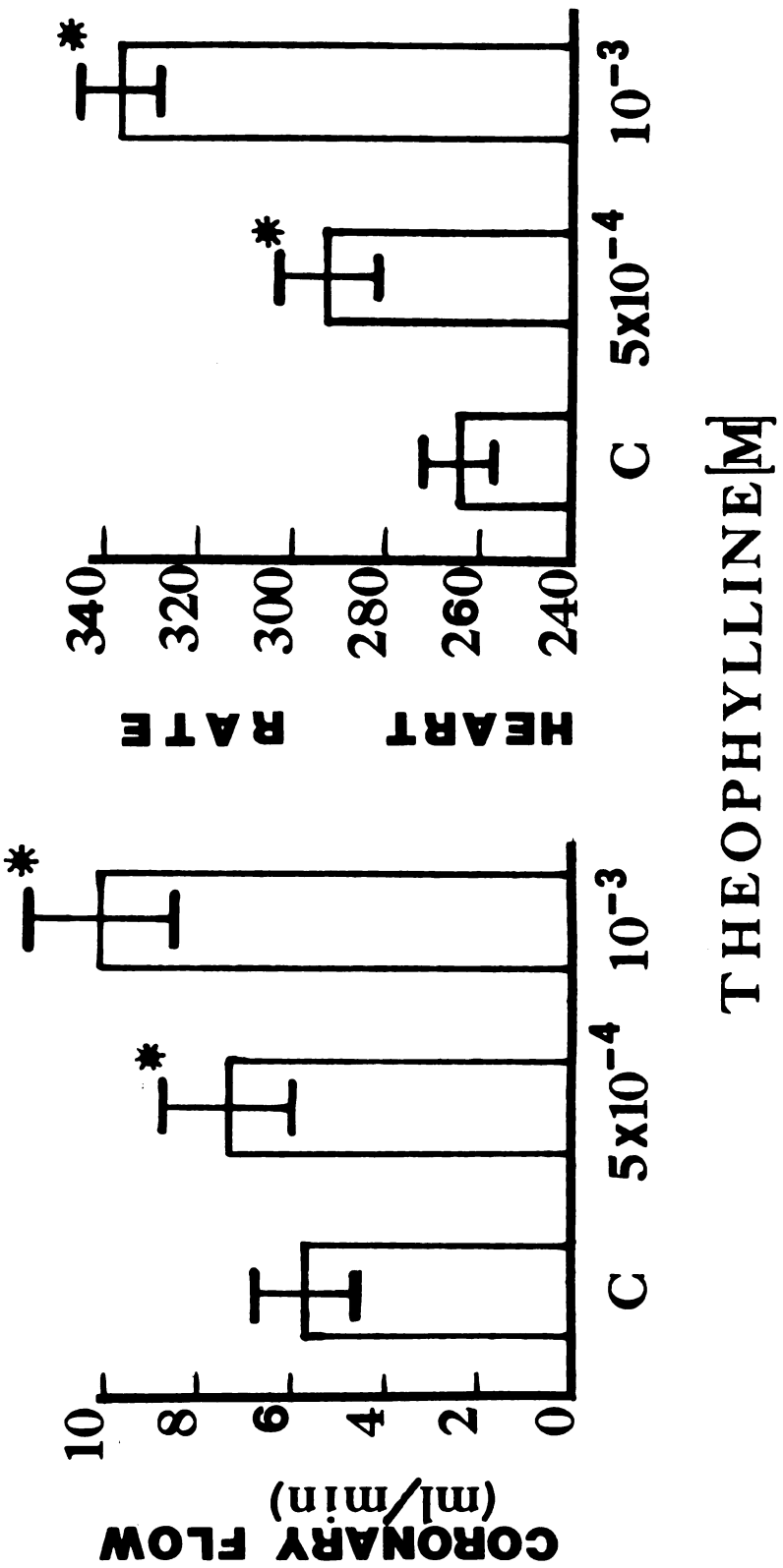

CORONARY FLOW
(ml/min)
10
8
6
4
2
0
C
5x10⁻⁴
10⁻³
HEART RATE
340
320
300
280
260
240
C
5x10⁻⁴
10⁻³
THEOPHYLLINE[M]

Figure 17

Table 8. Effect of theophylline on the coronary vasodilation produced by adenosine in perfusates with two different $[H^+]$. Hearts were initially perfused with solution of pH 7.43 followed by a perfusate of pH 7.20. (*) indicates statistical significance ($P < 0.05$) when compared to the response produced by adenosine. N = 9

		Coronary Flow (ml/min)		Heart Rate	
		pH = 7.43	pH = 7.20	pH = 7.43	pH = 7.20
	Pre-exper.	6.8±0.45	7.8±0.51	260±6.7	265±5.0
	Adeno. (8×10^{-7}M)	12.0±0.45	12.2±0.53	260±6.7	267±3.0
Theophylline	10^{-6}M	11.8±0.45	12.2±0.51	265±5.3	269±5.1
	10^{-5}M	10.8±0.60	11.6±0.60	270±4.9	271±5.8
	10^{-4}M	8.2±0.50*	9.8±-.60*	272±5.3	271±5.5
	Postexper.	6.8±0.50		269±12.9	

hearts with fresh solution, heart rate was still 15 beats/min above control but flow had returned to control levels.

VI. Perfusate pH and Theophylline's (10^{-6}-10^{-4}M) Capacity to Attenuate the Coronary Dilation Produced by Adenosine ($8x10^{-7}$M)

After evaluating the effect of theophylline on coronary flow at perfusate pH 7.43 and 7.20 we were ready to investigate the effect of lowering the perfusate pH on theophylline's ability to attenuate the coronary response to exogenous adenosine. Our goal was to use a concentration of adenosine which would produce marked but not maximal coronary dilation and to subsequently add to the reservoir quantities of theophylline which could successfully reduce the maximum flow produced by adenosine. Others (11) have previously shown that theophylline is a competitive antagonist of adenosine and that increasing adenosine above 10^{-6}M largely overcomes theophylline attenuation of the adenosine response. We wanted to see good theophylline attenuation so as to identify any effect of lowering the pH on the attenuation. Our previous experiments indicated that a good dilating concentration of adenosine could be found in the range $5x10^{-7}$-10^{-6}M. We chose $8x10^{-7}$M adenosine. This concentration of adenosine regularly increased coronary flow from 6.8±0.45 ml/min to a maximum of 12.0±0.45 ml/min in perfusate of pH 7.43 (Table 8, Figure 18). Theophylline

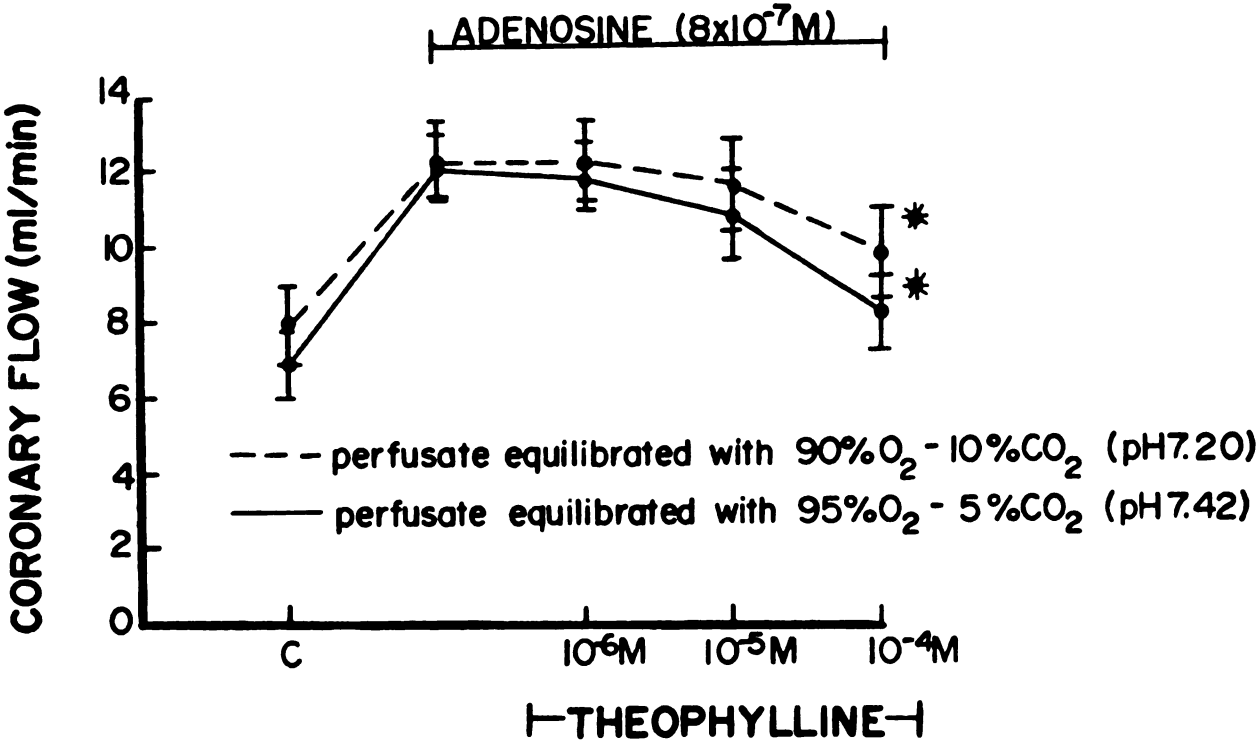


Figure 18: Effects of theophylline on coronary flow during the treatment with 8×10^{-7}M adenosine; hearts were first perfused with solution equilibrated with 95%O_2 - 5%CO_2 (pH7.42) N=9 * $p < 0.05$

(10^{-4}M) attenuated the response to $8x10^{-7}$M adenosine. The maximum coronary flow was 8.2±0.5 ml/min in perfusate of pH 7.43. When hearts were subsequently switched to a perfusate of pH 7.20, adenosine ($8x10^{-7}$M) produced an increase in flow similar to that seen at pH 7.43 in response to the same concentration of adenosine. Theophylline (10^{-4}M) again produced a significant ($P < 0.05$) effect by reducing flow, in the presence of $8x10^{-7}$M adenosine, from 12.2±0.53 to 9.8±0.6 ml/min.

Heart rate was only slightly increased by all concentrations of theophylline at each pH (Table 8).

VII. Attenuation of Adenosine ($8x10^{-7}$M) Dilation by Theophylline When Perfusing Initially with Solution of pH 7.20

Results from experiments presented in Figures 8 and 9 indicated that changing the order of perfusion (first perfusing with solution of pH 7.20 followed by solution of pH 7.43) had a marked potentiating effect on adenosine's capacity to dilate the coronary vessels. Thus, we wondered if theophylline's attenuating ability would be affected by reversing the order of perfusion.

In an attempt to answer this question nine hearts were initially perfused at pH 7.20 followed by perfusion at pH 7.43 (Table 9). Adenosine ($8x10^{-7}$M) increased coronary flow from 6.6±0.31 to 14.2±0.96 ml/min, and from 5.4±0.42

Table 9. Effects of theophylline on heart rate and adenosine-induced coronary dilation when hearts were initially perfused with solution of pH 7.20 followed by a perfusate with a pH of 7.43. All values are expressed as means ± S.E.M. (*) denotes significance ($P < 0.05$) when compared to the response produced by adenoeine. N = 9

		Coronary Flow (ml/min)		Heart Rate	
		pH = 7.20	pH = 7.43	pH = 7.20	pH = 7.43
	Pre-exper.	6.6±0.31	5.4±0.42	257±8.4	263±8.2
	Adeno. (8×10^{-7}M)	14.2±0.96	9.5±0.61	260±8.7	265±9.3
Theophylline	10^{-6}M	14.1±0.68	9.5±0.61	260±8.8	268±9.9
	10^{-5}M	12.8±0.35	8.6±0.70	263±7.7	272±9.9
	5×10^{-5}M	11.1±0.48*	7.6±0.71	266±6.4	270±9.4
	10^{-4}M	9.9±0.51*	7.1±0.62	269±7.6	268±9.8
	Postexper.		5.3±0.29	257±7.0	

to 9.5±0.61 ml/min respectively in perfusates of pH 7.20 and 7.43. Subsequent addition of theophylline (10^{-6}-10^{-4}M) did not significantly affect flow at pH 7.43 (Figure 19, bottom curve), even though in previous experiments the same concentration of theophylline at the same pH did produce significant attenuation (Figure 18). At pH 7.20, $5x10^{-5}$ and 10^{-4}M theophylline significantly ($P < 0.05$) reduced flow from 14.2±0.96 ml/min to 11.1±0.48 and 9.9±0.51 ml/min respectively.

In Figure 20 is shown the top curves of Figures 18 and 19, and it can be seen that at pH 7.20, regardless of perfusion sequence, theophylline (10^{-4}M) reduces coronary flow to the same level. Yet at pH 7.43, perfusion sequence did appear to affect the response to theophylline.

VIII. The Effect of Concurrent Theophylline, Ouabain and Alkalosis (Perfusate pH 7.69) on Reactive Dilation and Autoregulation

Several reports appear in which the effects of theophylline on reactive hyperemia (dilation) have been investigated. Others have studied ouabain's action on potassium dilation of the coronaries and have attempted to link the regulation of local blood flow to an action by potassium. Gerlach et al. (unpublished observation) found that ouabain ($1.4x10^{-7}$M) attenuated coronary dilation produced by hyperkalemia in the isolated perfused guinea pig heart.

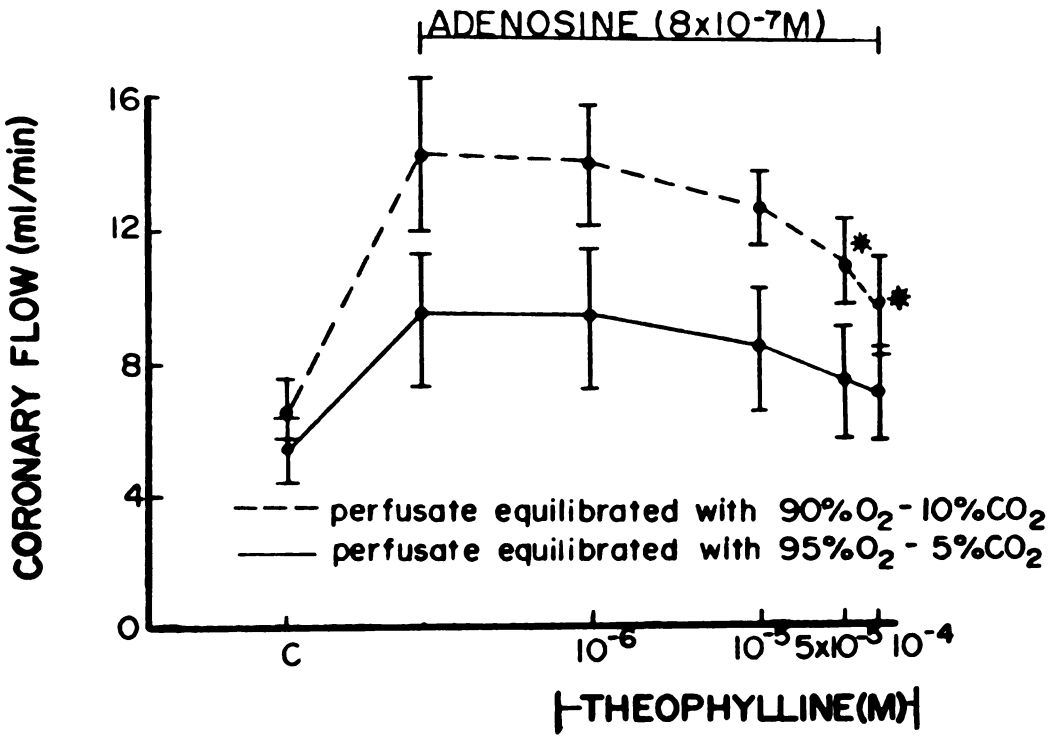


Figure 19: Effect of theophylline on coronary flow during treatment with 8×10^{-7}M adenosine. Hearts initially perfused with solution of pH 7.20.
* statistically significant from max., $p < 0.05$
data presented as mean ± S,E,M. N= 9

Figure 20. Effect of theophylline on the coronary dilation produced by adenosine ($8x10^{-7}M$). Data are from Figures 18 and 19. Upper curve shows coronary flow in hearts perfused first at pH 7.20 followed by pH 7.42. Lower curve presents flow values in hearts perfused initially at pH 7.42 and then at 7.20. (*) denoted statistical significance. ($P < 0.05$) when compared with the peak flow response produced by $8x10^{-7}M$ adenosine. Data points are the mean $\pm$ S.E.M. N = 18

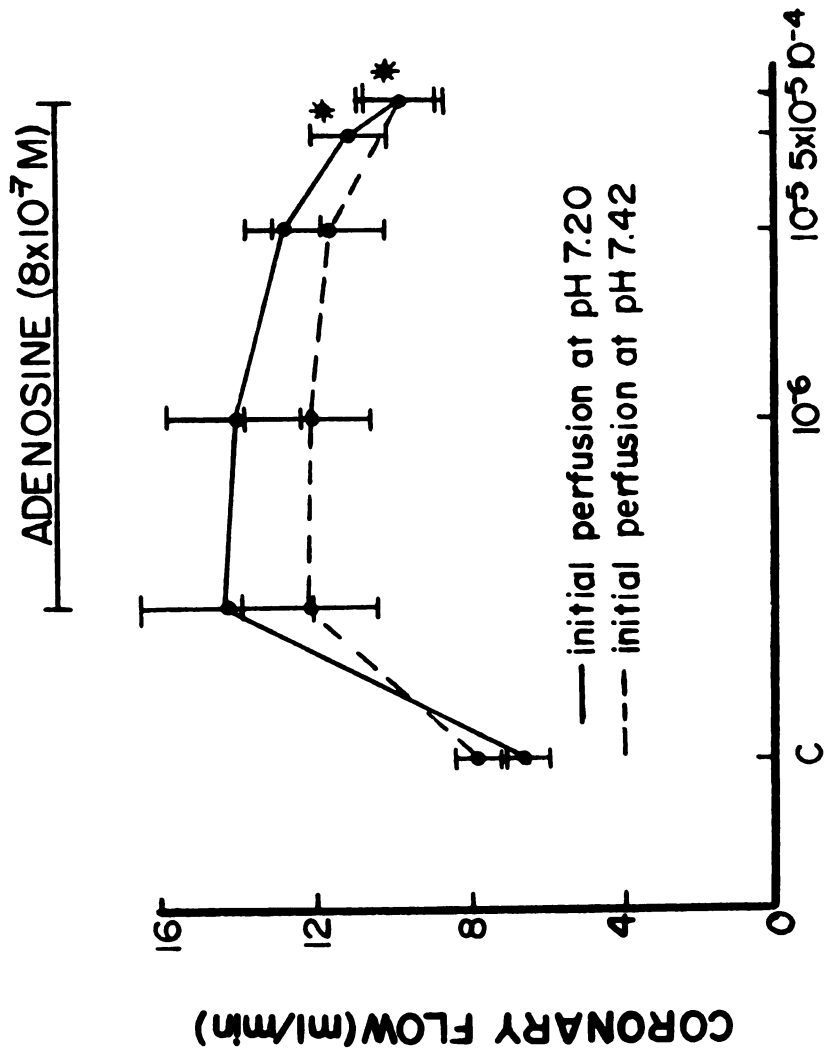

ADENOSINE (8x10⁻⁷M)
CORONARY FLOW (ml/min)
16
12
8
4
0
C
10⁻⁶
10⁻⁵
5x10⁻⁵
10⁻⁴
THEOPHYLLINE (M)
initial perfusion at pH 7.20
initial perfusion at pH 7.42

Figure 20

Hydrogen ion is also involved in controlling local blood flow and attempts have been made to evaluate its contribution to reactive hyperemia. However, no one to date has examined the effects on reactive hyperemia of trying to simultaneously attenuate the possible contributions of potassium, hydrogen ion and adenosine to this response. We have made an attempt to do this and our results are as follows.

Reactive dilation: Following release of a 30 second occlusion of the aortic inflow cannula, peak flow, volume of coronary flow for 20 seconds (VCF_{20}), and the time required for excess flow to return 50 per cent toward control (T_{50}) were examined in the absence and presence of concurrent theophylline, ouabain and alkalosis (perfusate pH 7.69). Control flow was not changed by these conditions. Results are presented in Table 10. Peak coronary flow, in the presence of theophylline, ouabain and alkalosis was slightly, but significantly reduced from 15.7±0.64 to 14.7± 0.55 ml/min. VCF_{20} and T_{50} were also reduced during experimental conditions, but failed to return to control values in the postexperimental state. Figure 21 shows a representative tracing of reactive dilation before (pre-experimental), during (experimental) and after (postexperimental) treatment with test agents, and illustrates that the changes seen in the presence of test agents were slight.

Table 10. Effect of concurrent theophylline ($5x10^{-5}$M), ouabain ($1.4x10^{-7}$ M) and alkalosis (perfusate pH 7.69) on coronary flow following release of a 30 sec. inflow occlusion. VCF_{20} = volume of flow in first 20 seconds following release of occlusion. T_{50} = time required from release of occlusion for the flow rate to return to 50% of peak flow. Values are presented as the mean ± S.E.M. (*) denotes statistical ($P < 0.05$) significance when compared to pre-experimental values. N = 8

	Peak flow (ml/min)	VCF_{20}(ml/min)	T_{50}(sec.)
Pre-experimental	15.7±0.64	3.7±0.15	13.9±0.40
Experimental	14.7±0.55*	3.2±0.19*	12.3±0.48*
Postexperimental	14.9±0.58	3.3±0.23*	11.9±0.72*

Figure 21. Effect of concurrent theophylline ($5x10^{-5}M$), ouabain ($1.4x10^{-7}M$) and alkalosis (perfusate pH 7.69) on reactive dilation following release of 30 sec. occlusion. Top and bottom panels show reactive dilation in the absence of test agents. N = 8

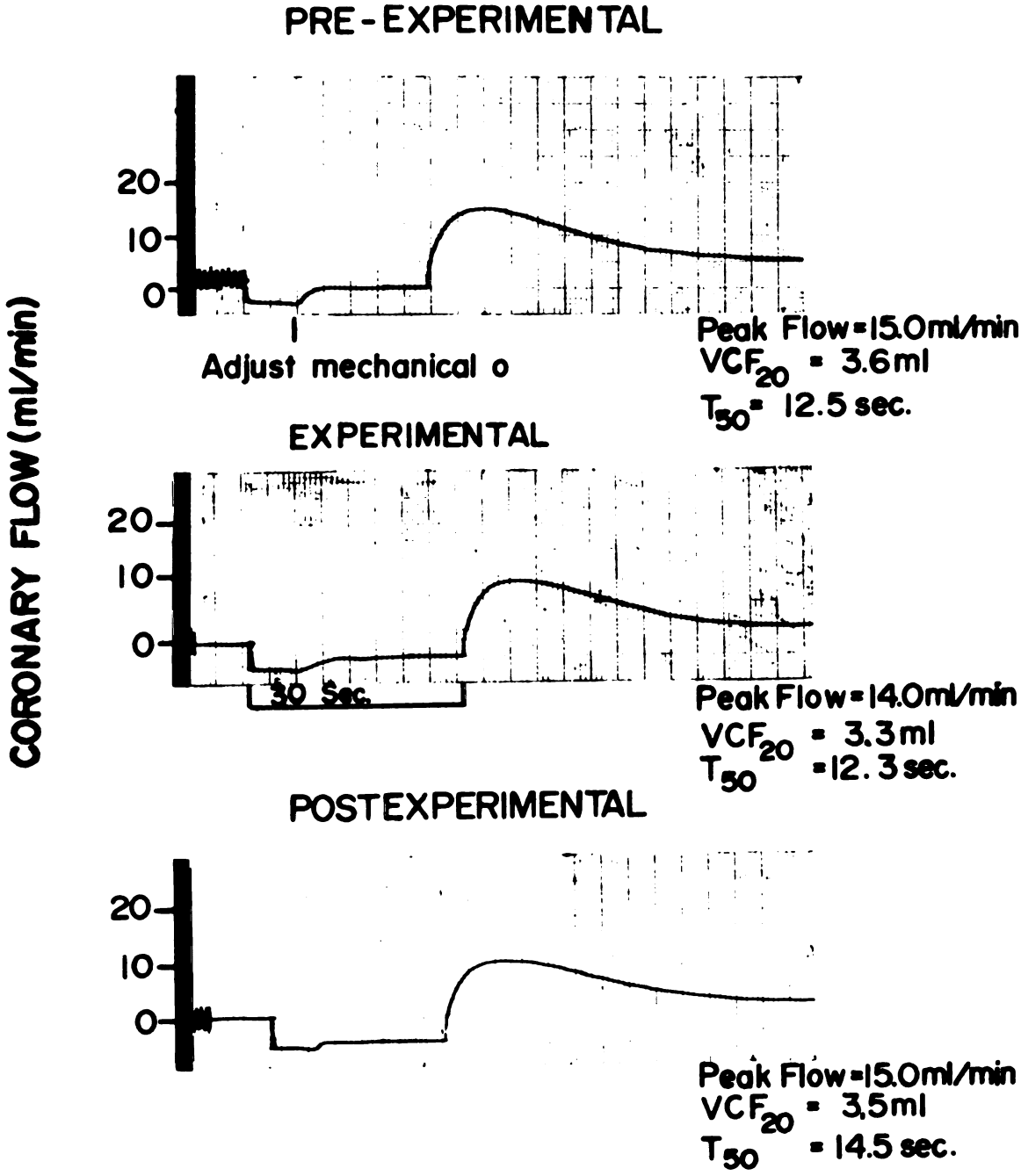

PRE-EXPERIMENTAL
CORONARY FLOW (ml/min)
20
10
0
Adjust mechanical o
Peak Flow = 15.0ml/min
VCF20 = 3.6ml
T50 = 12.5 sec.
EXPERIMENTAL
20
10
0
30 Sec.
Peak Flow = 14.0ml/min
VCF20 = 3.3ml
T50 = 12.3 sec.
POSTEXPERIMENTAL
20
10
0
Peak Flow = 15.0ml/min
VCF20 = 3.5ml
T50 = 14.5 sec.

Figure 21

Autoregulation: In the same group of hearts, the effects of theophylline, ouabain and alkalosis on autoregulation were investigated. Increasing perfuson pressure from 65 cm H_2O to 95 cm H_2O in the absence of test agents transiently increased flow from 6.3±0.41 to 11.4±0.61 ml/min; it then quickly fell to 8.5±0.57 ml/min. Calculated resistance to flow increased nonsignificantly from 10.3±0.68 to 11.2±0.76 cm H_2O/ml/min. The reservoir was then quickly dropped to a point which produced a perfusion pressure of 35 cm H_2O and flow decreased transiently to 1.4±0.37 ml/min, stabilizing at 4.4±0.13 ml/min. Calculated resistance decreased following the reduction of perfusion pressure to 8.0 cm H_2O/ml/min. Subsequently, the reservoir was raised to return the perfusion pressure to 65 cm H_2O. This produced a transient flow response typical of that seen upon release of a 30 second inflow occlusion.

The perfusate containing theohyplline ($5x10^{-5}M$), ouabain ($1.4x10^{-7}M$) and made alkalotic by equilibrating with 98% O_2-2% CO_2 (pH 7.69) was then perfused through the system (with no observable effect on coronary flow or heart rate) and the pressure-flow responses were again observed (Table 11, Figure 22). Coronary flow was increased from 6.1±0.44 to 9.7±0.53 ml/min and calculated resistance increased from 9.8±2.0 to 13.3±0.64 cm H_2O/ml/min when increasing perfusion pressure from 65 cm H_2O to 95 cm H_2O. Subsequent lowering

Table 11. Effect of concurrent theophylline (5×10^{-5} M), ouabain (1.4×10^{-7} M) and alkalosis on coronary flow (ml/min) and calculated resistance in response to increasing and decreasing perfusion pressure. * indicates statistical significance (P 0.05) when compared with respective control value. N = 8

Key: R = resistance (ml/min/cm H_2O
F = steady-state flow (ml/min)
P = peak flow at elevated pressure
M = minimum flow at lowered pressure
C = control
E = experimental (i.e., theophylline, ouabain and alkalosis)

65 cm H_2O				95 cm H_2O					
F		R		P		F		R	
C	E	C	E	C	E	C	E	C	E
6.3	6.1	10.3	10.6	11.4	9.7*	8.5	7.3*	11.2	13.0

35 cm H_2O					
M		F		R	
C	E	C	E	C	E
1.4	1.3	4.4	3.7*	8.0	9.5

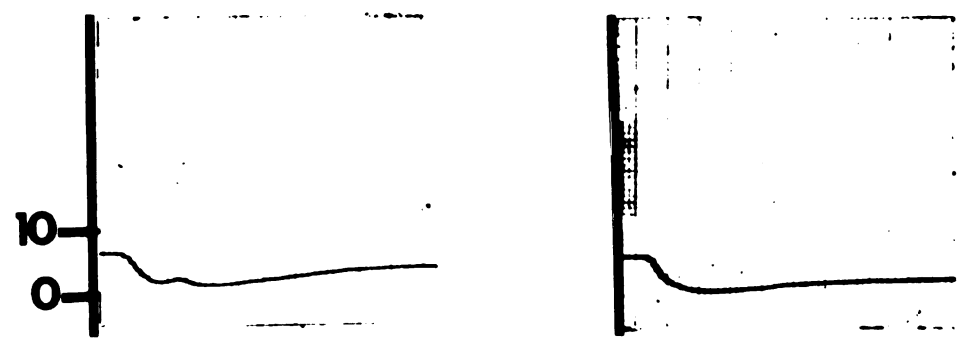
Figure 22. Effect of concurrent theophylline ($5x10^{-5}M$), ouabain ($1.4x10^{-7}M$) and alkalosis (perfusate pH 7.69) on coronary autoregulation. Left side shows responses in absence of test agents and right side shows responses in presence of test agents. N = 8

Increasing perfusion pressure from 65cm H_2O to 95cm H_2O.

Control Conditions

Experimental Conditions

Decreasing perfusion pressure from 95cm H_2O to 35cm H_2O.



CORONARY FLOW (ml/min)

Increasing perfusion pressure from 35cm H_2O to 65cm H_2O.

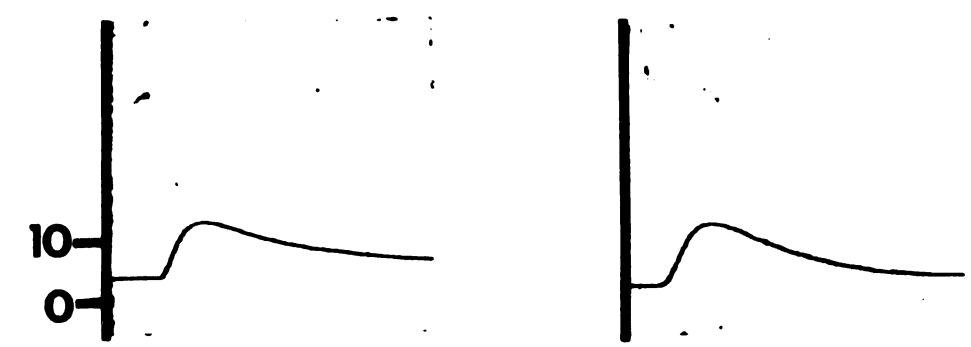


Figure 22

of perfusion pressure to 35 cm H_2O produced a transient reduction in flow, but flow quickly stabilized at 3.7±0.19 ml/min. Calculated resistance fell to 9.5±0.48 cm H_2O/ml/min which was significantly ($P < 0.05$) higher than that seen at the same pressure in the absence of test agents.

IX. Effect of Concurrent Theophylline ($5x10^{-5}M$), Ouabain ($1.4x10^{-7}M$) and Alkalosis (Perfusate pH 7.69) on Hypoxic Coronary Flow

In a group of seven hearts the coronary flow response to hypoxia was investigated in the absence of theophylline, ouabain and alkalosis. When coronary flow was stable under control conditions, hypoxia was induced by switching hearts to a previously prepared reservoir equilibrated with 20% O_2-5% CO_2 balance N_2. Mean flow rate was increased from 6.1 ± 0.52 ml/min to 14.1±0.83 ml/min. Diastolic flow and systolic flow were increased in a similar fashion (Figure 23). Hearts were then switched to fresh perfusate (pH 7.43), control data recorded (Figure 23, C_2) and subsequent perfusion with test solution containing theophylline ($5x10^{-5}M$), ouabain ($1.4x10^{-7}M$) and equilibrated with 20% O_2-2.5% CO_2 - balance N_2 was begun. Mean flow under these conditions increased from 6.0±0.54 ml/min to a maximum of 14.0±0.62 ml/min. There was no difference in the maximum mean flow achieved by hypoxia in the absence or presence of drugs. Diastolic flow in the presence of test agents

Figure 23. Effect of concurrent theophylline ($5x10^{-5}M$), ouabain ($1.4x10^{-7}M$) and alkalosis (perfusate pH) on coronary flow in response to hypoxia (perfusate equilibrated with 20% O_2-5% CO_2-balance N_2). P = presence of test agents, A = absence of test agents. All bars are means $\pm$ S.E.M. N = 7

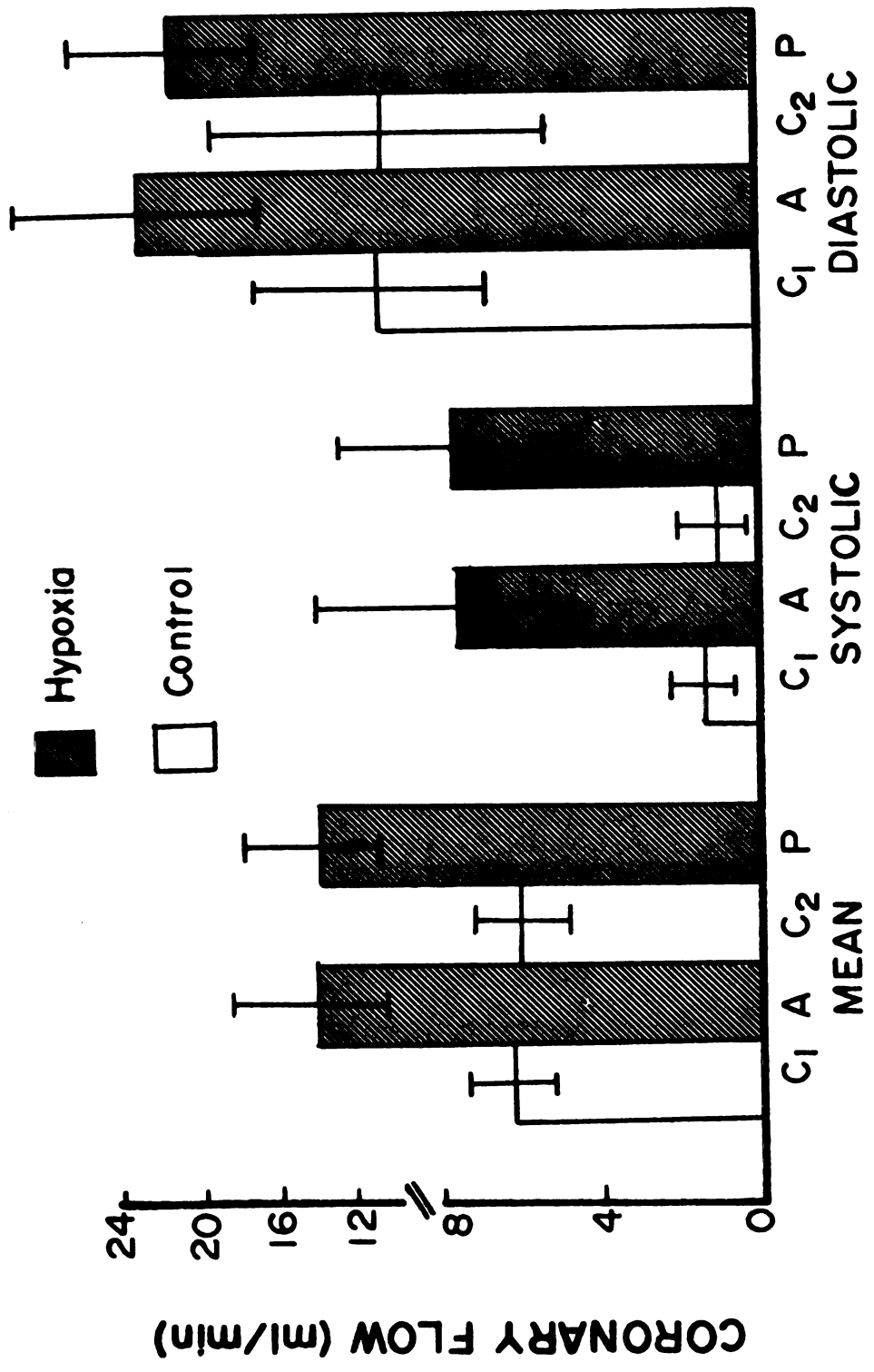

CORONARY FLOW (ml/min)
24
20
16
12
8
4
0
Hypoxia
Control
C1 A C2 P
MEAN
C1 A C2 P
SYSTOLIC
C1 A C2 P
DIASTOLIC

Figure 23

reached 21.1±0.74 ml/min as compared to 22.5±1.0 ml/min in their absence. Again statistical analysis revealed no significant difference.

DISCUSSION

Among the locally produced metabolites known to dilate the coronary vasculature, perhaps adenosine is the strongest contender for the role of metabolic mediator of coronary flow. However, for some time following the introduction of the adenosine hypothesis, investigators failed to find adenosine in coronary venous blood during ischemia and hypoxia. This was a source of criticism for the hypothesis because a physiologic mediator of blood flow must be present in the effluent during those conditions which evoke changes in blood flow. In addition, the proposed mediator must be present in quantities sufficient to produce the observed change in flow. With the improvement of experimental techniques it was soon shown that adenosine does appear in coronary venous perfusate during ischemia/hypoxia in quantities sufficient to produce the observed vasodilation.

Currently the adenosine hypothesis faces another source of opposition. Skepticism regarding the hypothesis exists since the finding (2,27,28,56,72) that aminophylline and theophylline fail to attenuate coronary reactive hyperemia and hypoxic coronary dilation, while successfully attenuating

coronary dilation by exogenous adenosine.

Work reported herein was aimed directly or indirectly at studying the possibility of an effect of perfusate hydrogen ion activity on adenosine's ability to dilate the coronaries, and/or on theophylline's ability to attenuate adenosine's coronary action. We investigated two major questions. The first being: Does altering the perfusate hydrogen ion activity affect the coronary adenosine dose-response relationship seen at perfusate pH 7.42? The second question was: Is the ability of theophylline to attenuate adenosine-induced coronary vasodilation pH sensitive? These questions are relevant to the observation that theophylline has little effect on coronary reactive hyperemia or coronary hypoxic dilation.

I. Adenosine Coronary Dilation as Affected by Perfusate Hydrogen Ion Concentration

Approaching the first question we compared the coronary dose-response curves for the effect of adenosine on the coronary vasculature in hearts perfused with solutions of different hydrogen ion concentrations. Several findings were of interest. We found that progressively lowering perfusate pH from 7.43 to 6.89 (in the absence of adenosine) produced an increase in flow which was significantly greater than the stable flow at pH 7.43. In none of these

experiments did altering the perfusate pH have an effect on spontaneous heart rate; therefore, the decrease in flow was attributed to a direct vasodilating action by hydrogen ion on the coronary vasculature. It is possible, however, that the increased flow seen when lowering the perfusate pH was not due to a direct action by hydrogen ion on the coronary vessels but rather was due to an indirect increase in transmural distending pressure subsequent to weakened cardiac contractile strength. No attempt was made to assess cardiac contractile activity so that the possible contribution to the increased flow produced by this mechanism is uncertain.

At pH 7.43, 7.36 and 7.20, 10^{-6}M adenosine produced rates of coronary flow which were not different from each other. However, when lowering the pH to 6.89, $5x10^{-7}$M adenosine produced an increase in coronary flow markedly greater than that achieved by 10^{-6}M adenosine at higher pHs. Statistical treatment of the data at pH 6.89 revealed an interaction between adenosine and perfusate pH in producing the observed response. That this enhanced response at pH 6.89 must be related to potentiation of either adenosine's or hydrogen ions actions is demonstrated by the fact that the coronary flow increase cannot be attributed to summation of the effects of hydrogen ion (pH 6.89) on coronary flow with the effects of adenosine ($5x10^{-7}$M; pH 7.42) on coronary flow. In other experiments the perfusate pH was

increased from 7.43 to 7.69, but coronary flow was not affected. If decreasing pH from 7.42 to 7.20 produced an acidosis which weakened contractile strength, yielding a passive increase in flow, one might argue that alkalosis could improve contractile strength, thus reducing coronary flow. When adenosine ($5x10^{-7}M$) was added to the perfusate at pH 7.69, both diastolic and mean flow increased to a much lesser extent than previously seen at pH 7.43 when the same adenosine concentration was used. Statistical analysis indicated a significant interaction between adenosine and hydrogen ion to produce this response. Thus, it is possible that the decreased hydrogen ion activity produced a direct reduction in adenosine's dilating action, or perhaps myocardial contractile strength was increased thereby decreasing the transmural pressure gradient and minimizing the adenosine-flow response.

How does one explain the fact that the perfusate hydrogen ion activity seems to potentiate adenosine's coronary action at a pH of 6.89 while lowering the hydrogen ion activity of the perfusate to a pH of 7.69 retards adenosine's ability to dilate the coronary vasculature? Recently Raberger, Weissel and Kraupp (73) reported from studies performed on an anesthetized dog, that adenosine's (intracoronary injection, 1-2 μg/kg) coronary dilator action correlated directly with the hydrogen ion concentration of the

blood. They found that this effect of adenosine was significantly dependent on arterial blood pH only if changes in systemic blood pH were accompanied by comcomitant changes in extracellular buffer capacity. A decrease in buffer capacity and pH (i.v. infusions of 0.1N HCl, 2 ml/min) led to an increase in adenosine's dilating action, while a rise in pH and extracellular buffer capacity (i.v. infusions of either 5% $NaHCO_3$, 5 ml/min, or tris-hydroxyamino-methane, THAM, 1M, 2 ml/min) resulted in a decrease. Eberlein (23) also reported that adenosine's coronary dilating action increased as arterial PCO_2 increased. Moreover, the effects of dipyridamole and hexobendline-coronary dilators with a potentiating effect on adenosine's coronary action (27,28,62) were found by Wolner and Kraupp (94) to be significantly dependent on the extracellular pH.

In the isolated perfused guinea pig heart we found that varying the pH of perfusing solution over the range of 7.69 to 6.89 in the presence of $5x10^{-7}$M adenosine resulted in changes in coronary flow not unlike those reported by Raberger et al. (73). As perfusate pH was decreased from 7.43 (control) to 6.89 a marked increase in coronary flow was produced which could not be accounted for by simply adding the individual effects on coronary flow regularly produced by pH 6.89 and by $5x10^{-7}$M adenosine. Subsequent statistical treatment of these findings revealed that

indeed lowering the pH to 6.89 produced a potentiation of adenosine's ability to dilate the coronaries. Moreover, increasing perfusate pH from 7.43 to 7.69 resulted in a reduction of adenosine's coronary dilating ability. Thus, although altering the pH was accomplished differently in the two studies, the results are similar and might be suggestive of a common mechanism of "interaction" by adenosine and hydrogen ion on the coronary vessels.

Further, Raberger et al. (73) reported other observations indicating that concurrent with adenosine's coronary action was an increase in intracellular lipid and carbohydrate metabolism accompanied by increased release of hydrogen ions and total CO_2 from the myocardial cells. Increased total CO_2 release from the myocardium would have an effect on extracellular buffer capacity similar to the infusion of acid in Raberger's study; both would reduce buffering capacity. Raberger postulated that the effect of adenosine on myocardial lipid and carbohydrate metabolism created a metabolic acidosis which was transferred to the coronary vasculature. When these investigators infused THAM (tris-hydroxyaminomethane) prior to HCl administration, they found that even though arterial pH did decrease, five times more HCl was required to drop the pH by 0.1 unit than was required when THAM was not administered prior to HCl. Also, in the THAM-pretreated experiments, arterial pH

shifted from 7.7 to 7.1 but adenosine's coronary dilator action was not enhanced. This was attributed to the fact that THAM entered the myocardial cells and buffered much of the hydrogen ion before it could be released from the cell. From this study they concluded that adenosine's coronary dilating action was mediated by an effect on myocardial metabolism as well as an effect on the coronary vasculature.

It seems reasonable to speculate that during myocardial ischemia and/or hypoxia there is an increased release of hydrogen ion from the tissues concomitant with an increased release of adenosine. The increase in hydrogen ion activity conceivably could affect appropriate coronary adenosine receptors thus increasing the vascular response to adenosine. Studies dealing with hydrogen ion activity in exercising skeletal muscle and those concerning adenosine's vascular action in this bed might be relevant to the speculation that concomitant release of adenosine and hydrogen ion from the myocardial cell during ischemia interact to dilate the coronary vessels. Rudke et al. (81) and Gollwitzer and co-workers (37) reported that associated with exercise hyperemia in active skeletal muscle is an increase in venous blood hydrogen ion activity. Although no attempt was made in these studies to assay for adenosine in the venous effluent, Berne and his co-workers (8) reported from constant-flow experiments on exercising skeletal muscle the

appearance of adenosine in venous blood coming from the exercising limb. They proposed that adenosine could be the mediator of the hyperemia associated with exercise. Additionally, Rubio et al. (78) were able to find adenosine in coronary venous blood during reactive hyperemia, but made no attempt to measure hydrogen ion activity. Thus, from such reports as those made by Rudko (81), Gollwitzer (37), Berne (8) and Rubio (78) it seems possible that a concomitant release of adenosine and hydrogen ion from exercising skeletal muscle and/or from stressed cardiac muscle could produce an increased blood flow that is due to potentiation of adenosine's actions by hydrogen ion.

Other possibilities could account for the apparent enhanced coronary action of adenosine at a low pH. Evidence indicates that cardiac contractile strength is weakened by an acidotic perfusate (59). Decreased extravascular compression of the coronaries resulting from weakened contractile strength would result in a net increase in coronary transmural pressure (passive dilation). An increase in coronary blood flow could accompany such an action. Fuchs et al. (33) recently found that hydrogen ion inhibits the binding of Ca^{2+} by troponin in cardiac muscle, and Katz (59) reported evidence that a fall in intramyocardial pH, as in ischemic myocardium, might be directly responsible for a reduction in contractile strength.

More speculative mechanisms for the increased response to adenosine at a low pH could include an inhibitory action by the increased hydrogen ion activity on enzymes responsible for the degradation and/or rephosphorylation of adenosine. Such an action would possibly result in greater tissue concentrations of adenosine. In keeping with such a possibility is the report by Jacob and Berne (49) that adenosine deaminase (the enzyme responsible for the deamination of adenosine to inosine) and nucleoside phosphorylase (an enzyme capable of rephosphorylating adenosine to yield AMP) are leached out of the isolated, perfused cat heart during recirculation of perfusate.

Based on the assumption that during myocardial ischemia and/or hypoxia a concomitant release of adenosine and hydrogen ion occurs, our finding that hydrogen ion potentiates adenosine's coronary dilation could possibly explain, in part, theophylline's inability to block reactive hyperemia. It is conceivable that increased hydrogen ion activity in the vicinity of the coronary adenosine "receptor" helps adenosine more effectively dilate the coronaries thus precluding theophylline attenuation.

In the experiments discussed thus far, the coronary action of adenosine was always initially examined in a perfusate of pH 7.43 followed by subsequent perfusion at a pH of either 7.36, 7.20 or 6.89. We therefore deemed it

necessary to "reverse the sequence of perfusion," i.e., assess adenosine's coronary effects first at pH 7.20 then switch to a perfusate of pH 7.43 and again treat with adenosine. This seemed important for the sake of detecting a patterned adenosine coronary response which might have resulted from the experimental bias of always perfusing hearts at pH 7.43 initially. Figure 8 presents data from experiments in which coronary vessels were perfused first with solution of pH 7.20 followed by perfusion at a pH of 7.43. Comparison of the results shown in this figure with results presented in Figure 5, in which coronary vessels were perfused initially at pH 7.43 followed by perfusion at pH 7.20, reveals some marked differences. We discovered that 10^{-6}M adenosine in a perfusate of pH 7.20 produced a much greater response in hearts perfused first at pH 7.20 than in those hearts perfused first at pH 7.43 followed by perfusion at pH 7.20. Note that the portion of the curve (Figure 8) at pH 7.20 from 10^{-8}M adenosine and beyond has been shifted up and to the left. This indicates that at a low pH less adenosine is required to produce a given coronary response, or alternatively, increasing perfusate pH to 7.42 competitively antagonized the ability of adenosine to dilate the coronary vasculature. At pH 7.42, ten times as much adenosine was needed to produce a coronary response similar to that produced by 10^{-7}M adenosine at pH 7.20.

Hence, this finding suggests that adenosine might be acting at a coronary receptor site which is sensitive to hydrogen ion. Statistical analysis of the coronary response to adenosine in hearts perfused first at pH 7.20 suggests that the difference in adenosine's action from that at pH 7.20 in hearts first perfused with solution of pH 7.43 results from potentiation by hydrogen of the adenosine action in the former (Appendix A, Table A5). The only difference in protocols of experiments presented in Figures 5 and 8 is that of reversing the order of perfusion heretofore used, i.e., we perfused initially with a perfusate of a pH other than 7.43.

In our early experiments in which hearts were first perfused with solution of pH 7.43, a new perfusate with a lower pH was not introduced for some 30 to 45 minutes following initial stabilization of coronary flow. During this period of time, the hearts probably achieved a steady state between the metabolic production of hydrogen ions and CO_2 and the ability of the $NaHCO_3$ in the perfusate to buffer. Conversely, hearts first treated with solution of pH 7.20 were not allowed the additional 30 to 45 minutes to achieve stable buffering conditions. Consequently, the ability of $NaHCO_3$ to buffer the increased hydrogen ion of the perfusate in addition to that released from the myocardium might have been temporarily overwhelmed, allowing hydrogen

ion concentration to increase and resulting in significant potentiation of adenosine's coronary action.

II. Adenosine's Coronary Action at a Low pH for an Extended Period of Time

Examination of the results from experiments in which hearts were initially perfused with solution of pH 7.20 followed by perfusion at pH 7.43, revealed that the coronary response to $5x10^{-7}$M and 10^{-6}M adenosine at pH 7.20 were not statistically different and that at 10^{-6}M, the increase in coronary flow was reaching a plateau.

A steady level of coronary flow at pH 7.20 in response to $5x10^{-7}$-10^{-6}M adenosine raised the question of whether or not adenosine can maintain an increased coronary flow over an extended period of time at a low pH. An answer to this question seemed pertinent to the adenosine hypothesis for if adenosine is to be assigned a significant role in producing and maintaining the increase in coronary flow accompanying prolonged exercise, it must be shown that adenosine's coronary dilating action does not diminish during periods of heavy myocardial work. Additionally, this experiment served as a means of testing if attenuation of the adenosine coronary response by 10^{-4}M theophylline (from other experiments in this study) was partially due to a waning of adenosine dilation with time. The finding that adenosine dilation at

a low pH is maintained for 25-30 minutes is interesting. Adenosine has been postulated not only as the mediator of coronary blood flow, but of skeletal muscle blood flow during exercise (8). Additionally, it is known that the pH of venous effluent blood from active skeletal muscle decreases (37,81) during exercise. With periods of increased myocardial oxygen demand, coronary flow increases and adenosine has been found in the coronary venous effluent (78,79). If myocardial tissue pH and/or coronary venous effluent pH decrease under conditions of increased oxygen demand, it would be tempting to speculate that the low pH coupled with simultaneous adenosine release interact to produce the increased flow in both of these vascular beds. To my knowledge, the ability of adenosine to maintain an increase in skeletal muscle blood flow has not been studied over an extended period of time. Again, statistical evaluation of this response suggested that adenosine's action was being enhanced by the hydrogen ion concentration of the perfusing fluid and that under these conditions of low pH adenosine is capable of maintaining elevated coronary flow for at least 25 minutes.

III. Effect of Variable Perfusate pH on the Coronary Action of a Single Concentration of Adenosine

Varying the pH of perfusate stepwise between 7.69 and 6.89 as well as randomizing the order of perfusion at different pHs produced a marked increase in coronary flow. When adenosine ($5x10^{-7}M$) was subsequently added to the perfusate, we found that its dilating action when compared to that at pH 7.43, was markedly enhanced by perfusates with a low pH and noticeably diminished at pH 7.69. Thus, regardless of whether our studies dealt with a range of adenosine concentrations at a constant perfusate pH, or if they assessed the action of a single concentration of adenosine over a range of perfusate pHs, the coronary dilating capacity of adenosine was affected similarly by the hydrogen ion activity of the perfusing fluid; decreasing the hydrogen ion activity inhibited adenosine's coronary actions and increasing the hydrogen ion activity enhanced adenosine's coronary action.

An interesting sidelight of the experiments in which the coronary action of a single concentration of adenosine was investigated over a range of pHs is the finding that upon reducing the perfusate pH to 6.89, the extent of coronary dilation produced by adenosine ($5x10^{-7}M$) was virtually equal to that produced by a 250 μg bolus of adenosine at pH 7.43. Others (11) have reported that in the isolated

perfused guinea pig heart maximum coronary dilation is produced by a bolus of 250 μg of adenosine. Another report (78) indicates that infusion of 56 nmoles/100 ml adenosine produces maximum coronary dilation in the dog. Both of these concentrations are much greater than $5x10^{-7}M$ used in our experiments.

Thus, several of our findings concerning adenosine coronary dilation as affected by changes in the perfusate pH, have shown that increasing the hydrogen ion activity in the perfusing fluid enhances adenosine's dilating ability. It therefore seems reasonable that if the tissue pH decreased during reactive hyperemia and/or hypoxia, theophylline's failure to block these responses might be partially explained on the basis that the increased hydrogen ion activity enhances adenosine dilation of the coronary vasculature thereby precluding theophylline attenuation.

IV. Theophylline Attenuation of Adenosine Coronary Dilation as Affected by Perfusate pH

Using concentrations of theophylline that produced no observable change in coronary flow, we sequentially attenuated the increase in coronary flow produced by $8x10^{-7}M$ adenosine and found that in hearts perfused initially with solution of pH 7.43 followed by perfusion at pH 7.20, theophylline's (10^{-6}-$10^{-4}M$) ability to attenuate adenosine's

coronary dilation was virtually unaffected by the hydrogen ion concentration of the perfusate. When we subsequently conducted experiments in which hearts were initially exposed to perfusate of pH 7.20 and were later perfused at pH 7.42 we found that the total absolute reduction in flow produced by theophylline at pH 7.20 appeared to be greater than that seen in previous experiments at pH 7.20 or 7.42 by the same concentration of theophylline. However, this apparent difference in effects by theophylline is not interpreted as being a "potentiation," by the increased hydrogen ion concentration, of theophylline's capacity to block adenosine at a low pH, but rather can be explained on the basis that at pH 7.20 adenosine was more effectively dilating the coronary vessels, thus providing theophylline with a larger flow to attenuate. When hearts were perfused at pH 7.42 first and then switched to pH 7.20, theophylline (10^{-4}M) was successful at both pHs in attenuating the adenosine response. However, in hearts which were immediately switched to perfusate of pH 7.20 following stabilization, theophylline (10^{-4}M) at pH 7.42 failed to significantly attenuate the adenosine response (Results, Section VII, Figures 18 and 19). Tables 8 and 9 indicate that adenosine's dilating action at pH 7.43 under these two sets of conditions was different. Table 9 shows that adenosine ($8x10^{-7}$M) failed to dilate as well at pH 7.43 when hearts were first exposed to perfusate

of 7.20 as compared to its dilator action at pH 7.43 in hearts first perfused at pH 7.43 (Table 8). This might suggest that under the former conditions, less adenosine was binding to respective receptors, thus providing a reduced amount of flow for theophylline to attenuate. In the latter condition perhaps more receptors were activated by adenosine thus providing a greater amount of adenosine-induced flow for theophylline to competitively antagonize. We thus concluded from these results that the inability of theophylline to block reactive hyperemia is probably not affected by an increased hydrogen ion activity in the blood. Had theophylline's ability to attenuate adenosine coronary dilation been diminished at the low pHs, then the failure of theophylline to attenuate reactive hyperemia might have been explained.

V. Coronary Reactive Hyperemia as Affected by Theophylline and Perfusate pH

Any contribution by adenosine to coronary reactive hyperemia must be reconciled with reports that theophylline, a competitive inhibitor of adenosine (10,11), fails to block, consistently, reactive hyperemia (9,27,28,55,56). Several investigators (15,78,91) have suggested that some of the conflict concerning theophylline's inability to regularly block reactive hyperemia is attributable to the ways in which investigators variously characterize reactive

hyperemia (9,15,56,78). Others have proposed (15,84) that tissue concentrations of adenosine during occlusion of the coronary vessels reach levels which can not be successfully blocked by theophylline. Still there are those (84) who argue that usable concentrations of theophylline (those which do not exert cardiotonic actions) are too weak to block coronary reactive hyperemia.

We have found that in the presence of $5x10^{-5}M$ theophylline and at pH 7.43, peak reactive dilation resulting from a 30 second inflow occlusion was not reduced when compared to a control response in the absence of theophylline. However, peak flow may not be a good index of reactive hyperemia as evidenced by the study of Curnish and coworkers (15). They found that aminophylline, while without a measurable effect on peak reactive hyperemia, reduced the volume of reactive hyperemic flow, and the duration of reactive hyperemia by 42 and 31 per cent respectively. In our study we found that in the presence of $5x10^{-5}M$ theophylline, the total area under the diastolic hyperemic curve (quantitated by planimetry in sq. cm) was only reduced by 8 per cent as compared to control, and that T_{50} was not affected. Also studying reactive hyperemia, Wadsworth (91) found that in the presence of aminophylline (10 mg/kg i.v.) the duration of reactive hyperemia was noticeably reduced when compared to control responses in the

absence of aminophylline. Conversely, Eikens and Wilcken (27.28) reported that in their studies, aminophylline (10 mg/kg, slow i.v. injection) failed to affect either the duration of reactive hyperemia or the volume of excess flow following release of 4-, 8- and 60 second occlusions. Wadsworth's experiments were performed on anesthetised cats, and reactive hyperemia was studied by occluding the left anterior descending coronary artery for 30 seconds. Eikens and Wilcken studied unanesthetised greyhounds when occluding for 4 and 8 seconds, but used anesthetised mongrels when studying responses to 60 seconds of occlusion. In Eikens and Wilckens studies they found no qualitative differences in reactive hyperemic responses in the presence and absence of aminophylline whether animals were anesthetised or unanesthetised. Perhaps in our experiments and in those of Curnish et al. (15), peak flow was not affected because it is more strongly influenced by the vascular distending force produced by the sudden surge of perfusate as the inflow occlusion is released, while the duration and volume of flow might be more strongly affected by vasodilator metabolites, and are therefore more susceptible to blockade by theophylline/aminophylline.

Later, when attempting to attenuate the possible contribution of adenosine, potassium and hydrogen ions to reactive dilation, we found that the concurrent presence of

theophylline ($5x10^{-5}M$), ouabain ($1.4x10^{-7}M$) and alkalosis (perfusate pH 7.69) produced a small but significant effect on peak flow following release of the occlusion, and was responsible for a slight but significant reduction in the volume of coronary flow and in the time required for flow to return half way toward control. Several minutes after perfusing with fresh solution, coronary inflow was similarly occluded and upon release it was found that the volume of coronary flow and time for flow to return toward control were still reduced. Hence it was concluded that the reduction in responses during the postexperimental state was due to the fact that test agents were still present or conversely, that the slight reduction in volume of flow and in time for flow to return toward control in the experimental state was due to something other than an effect by theophylline, ouabain and alkalosis. No attempt was made to study, individually, the possible contributions of potassium and hydrogen ions to the reactive hyperemic response.

VI. Effect of Concurrent Theophylline, Ouabain and Alkalosis on Hypoxic Coronary Dilation

Others (2,91) have been unsuccessful in blocking, with theophylline/aminophylline, coronary dilation produced by hypoxia. In an effort to minimize possible contributions by adenosine, potassium ions and hydrogen ions to the coronary

response to hypoxia, we added theophylline and ouabain to an hypoxic perfusate and made it alkalotic by reducing the concentration of carbon dioxide. The response of the coronary bed under these conditions was compared to a similar coronary response produced by hypoxia in the absence of test agents and no difference was found. It appears that with the particular blocking agents used in this experiment, in conjunction with the degree of hypoxia produced, our results fail to support but do not rule out a role by adenosine, potassium ion and hydrogen ion in producing the coronary dilation accompanying hypoxia. It is possible that tissue levels of adenosine during hypoxia were not blockable by the concentration of theophylline used in this experiment. Bunger et al. (11) have recently reported that theophylline is a competitive antagonist of adenosine and that theophylline attenuation of adenosine coronary dilation can be greatly overcome by increasing the concentration of adenosine in the perfusate. Failure by Afonso and co-workers (2) in the dog, and by Wadsworth (91) in the anesthetized cat to block hypoxic coronary dilation with aminophylline led these investigators to conclude that adenosine is probably not involved in producing the observed coronary hyperemia. Conversely, Scott et al. (84) have found that 10^{-3}M theophylline, a concentration much greater than can be used to block coronary hypoxic/reactive hyperemias in

the isolated perfused heart, is effective in abolishing renal vasoconstriction produced by perfusing an isolated, denervated bioassay kidney with hypoxic coronary sinus blood from a donor dog. Additionally, theophylline (10^{-3}M) blocked renal vasoconstriction produced by injection of adenosine (10-20 μg) into the renal artery of the bioassay kidney.

VII. Effect of Concurrent Theophylline, Ouabain and Alkalosis on Coronary Autoregulation

Ono and co-workers (71) reported that pretreatment of the renal vascular bed with theophylline-ethylenediamine (aminophylline) blocked the kidney's ability to autoregulate. We compared the ability of the coronary bed to autoregulate in the presence of concurrent theophylline ($5x10^{-5}$M), ouabain ($1.4x10^{-7}$M) and alkalosis (perfusate pH 7.69) with its ability to autoregulate in the absence of these test agents. Upon raising perfusion pressure from 65 cm H_2O to 95 cm H_2O we found that in the presence of test agents both peak flow and steady state flow at the elevated pressure were significantly reduced while calculated resistance was increased. In explaining these results two factors must be considered: as pressure is elevated, the increased transmural distending pressure should elicit myogenic constriction of the coronary

vasculature. Additionally, the increase in pressure produced an increase in coronary flow which should enhance washout of adenosine, potassium ions and hydrogen ions, thus favoring a return of flow towards control. However, the coronary dilating action of the unblocked portion of these chemicals would tend to dilate the coronary vessels. The effect of test agents, both during the transient increase in flow accompanying sudden elevation of perfusion pressure and during the new steady state flow, appears to have reduced the relaxing effects of adenosine, potassium ion and hydrogen ion on coronary vessels.

Subsequent lowering of hydrostatic pressure from 95 cm H_2O to 35 cm H_2O, produced, in the presence of theophylline, ouabain and alkalosis, a significant reduction in the ability of the coronary vessels to readjust flow toward control. It is reasonable to assume that at 35 cm H_2O perfusion pressure, coronary flow is still related to a composite interaction of myogenic smooth muscle activity and the opposing actions of vasodilator metabolites. Thus in the presence of test agents, the relaxing effect of adenosine, potassium ion and hydrogen ion on smooth muscle is effectively reduced and the coronary vasculature is less capable of autoregulating flow (Table 11). It is difficult to attribute the effects seen at a high or at a low perfusion pressure (in the presence of test agents) to an individual agent, nor can a proportionate contribution be assigned to each.

SUMMARY

The possibility that adenosine, a vasodilator, is a significant contributor to coronary reactive and hypoxic hyperemias was central to work reported in this study. Theophylline, a competitive antagonist of adenosine, attenuates the coronary response to exogenous adenosine but does not greatly affect the magnitudes of reactive and hypoxic hyperemias. Since in states of increased cardiac metabolism the tissue hydrogen ion activity might be increased, this study deals with the possibility that theophylline's ineffectiveness in attenuating reactive hyperemia and hypoxic dilation might be related to an interaction of hydrogen ion with adenosine and/or theophylline.

We found that lowering perfusate pH by increasing the PCO_2 had a statistically significant potentiating effect on adenosine dilation of the coronary vessels at a pH below 7.0 as compared to adenosine's action at pH 7.43. Conversely, it was found that increasing the pH of the perfusing fluid to 7.69 inhibited adenosine's ability to dilate the coronary vasculature. Also in hearts switched to a perfusate of pH 7.20 following stabilization, the coronary response to adenosine appeared to be enhanced. To account

for the increased coronary flow produced by adenosine at a low perfusate pH several possible mechanisms are considered, including 1) an interaction of hydrogen ion with adenosine to enhance coronary dilation, 2) weakening of contractile strength by the acidotic perfusate with a subsequent increase in coronary transmural distending pressure, and 3) the possible interference by acidosis of the enzymatic degradation and/or rephosphorylation of adenosine.

We also investigated the coronary response to adenosine at a low pH for an extended period of time (30 min). This experiment warranted attention for two reasons. First, it served as a 'check' on experiments in which theophylline was used to attenuate the coronary dilation produced by adenosine. We wanted to ensure that theophylline attenuation was not partially due to waning of the adenosine response. Secondly, if adenosine is to be assigned an important role in the regulation of coronary flow during prolonged exercise, it must be shown that adenosine's dilating action does not diminish with time. We found that 25 minutes after maximal dilation was achieved by 8×10^{-7}M adenosine, coronary flow was still 99 per cent of the maximal response and 159 per cent greater than control. Thus, adenosine is capable, at a low pH, of sustaining an increased coronary flow for an extended period of time.

Theophylline was without effect on coronary flow and spontaneous heart rate in concentrations up to 10^{-4}M, although at higher concentrations ($5x10^{-4}$ and 10^{-3}M) coronary flow and heart rate were both increased significantly. Other hearts were used to see if theophylline attenuation of the adenosine response is pH sensitive. Upon reducing perfusate pH to 7.20 it was found that vasoinactive concentrations of theophylline attenuated the adenosine response as effectively as seen at pH 7.42. Further, in hearts initially perfused at pH 7.20 (following stabilization at pH 7.42), adenosine increased coronary flow to a greater degree than was normally seen but theophylline was still able to effectively attenuate this response.

Theophylline, $5x10^{-5}$M, was essentially without effect on the reactive hyperemia seen following 30 seconds of coronary inflow occlusion. Since the potassium and hydrogen ions have also been suggested as mediators of hypoxic and ischemic dilation and of autoregulation, ouabain ($1.4x10^{-7}$M), a blocker of potassium vasodilation, and alkalosis (perfusate pH 7.69) were combined with theophylline ($5x10^{-5}$M) in an attempt to attenuate these manifestations of local regulation by minimizing the contribution of potassium and hydrogen ions and adenosine. Hypoxic dilation was unaffected. Both the volume and duration of hyperemic flow following release of a 20 second inflow

occlusion were reduced but failed to return to control after normalizing the perfusate. Peak reactive dilation was not affected. It is hard to determine if the reduced responses in the presence of test agents were effected by these agents. In previous experiments any effect by theophylline or hydrogen ion disappeared within a few minutes of perfusate normalization. In view of its persistent actions it is conceivable that ouabain could have accounted for the effect noted. The ability of hearts to autoregulate still occurred.

These studies suggest that an increase in hydrogen ion concentration increases and a decrease in hydrogen ion concentration decreases adenosine's coronary dilating ability. They also show that reducing perfusate pH has little effect on theophylline's ability to attenuate adenosine coronary dilation. Further, adenosine, at a low pH, has the ability to maintain coronary dilation for an extended period of time. If, in fact, adenosine is more active in the presence of acidosis, this could in part explain why theophylline fails to greatly modify reactive and hypoxic hyperemias.

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APPENDICES

APPENDIX A

STATISTICS AND ANOVA TABLES

STATISTICS USED

The nature of the adenosine and theophylline dose-response studies made their analysis applicable to the use of a RCB analysis of variance (two-way ANOVA) using factorials. Factorial analysis of variance is the test of choice when dealing with studies in which two or more variables (such as adenosine and perfusate pH) might interact in producing a common response (83). The majority of the studies presented herein are based upon an interaction of the variables involved. Analysis of variance, however, is merely the initial means of testing for data variance. In order to subsequently recognize differences in control and experimental means, several tests were used. If in the initial analysis of variance the treatment effect was significant (calculated F greater than tabular F), Tukey's procedure, i.e., LSR (least significant range test) was used to determine statistical difference between/among means (R. R. Sokal and F. J. Rohlf, Biometry) (83). If, however, the initial variance ratio test was not significant (calculated F less than tabular F), then an a priori test, LSD (least significant difference) was used to test for mean differences. If the design of the experiment was not

concerned with interaction of main effects, the Student t test modified for paired replicates was used to answer the question: Is the mean difference ($\bar{d}$) amongst pairs significantly different from zero?

In other experiments a regression analysis (method of least squares), was applied to the data to determine if the dependent variable was significantly regressed on the independent variable. Using the method of least squares, linearity of the regression curve is assumed; however, an analysis of variance was always applied to test for linearity.

Appendix A presents ANOVA tables from appropriate experiments.

Analysis of Variance (ANOVA) Tables computed from experiments in which the effect of adenosine on coronary flow was examined at perfusate pH 7.42 vs 7.36 (Table A1), 7.42 vs 7.20 (Table A2), 7.43 vs 6.89 (Table A3), 7.43 vs 7.69 (Table A4), and 7.43 vs 7.20 when hearts were initially treated with perfusate of pH 7.20 (Table A5).

Key: df = degrees of freedom
SS = sum of squares
MS = mean square
* = significance at α 0.05
** α = 0.001

Table A1. ANOVA (2x4 Factorial)

Source	df	SS	MS	Cal.F	Tab.F
Blocks	8	33	4.13	*3.72	1.97
Treatment	7	219	31.3	*28.2	2.51
perfusate pH	1	17	17	*15.3	5.29
adenosine	3	196	65.3	*58.8	3.34
interaction	3	6	2	1.80	3.34
Error	56	62	1.11		
Total	71				

Table A2. ANOVA (2x4 Factorial)

Source	df	SS	MS	Cal.F	Tab.F
Blocks	8	41.2	5.15	*3.06	1.97
Treatment	7	376	53.7	*32.0	2.51
perfusate pH	1	76.6	76.6	*45.6	5.29
adenosine	3	284	94.7	*56.4	3.34
interaction	3	15	5	2.98	3.34
Error	56	94	1.9		
Total	71				

continued

Table A3. ANOVA (2x5 Factorial)

Source	df	SS	MS	Cal.F	Tab.F
Blocks	5	27	5.4	*4.86	2.85
Treatment	9	414	46	*41.4	2.41
perfusate pH	1	130	130	**117	11.4
adenosine	4	210	52.5	*47.3	3.07
interaction	4	74	18.5	*16.7	3.07
Error	45	50	1.11		
Total	59				

Table A4. ANOVA (2x5 Factorial)

Source	df	SS	MS	Cal.F	Tab.F
Blocks	7	429	61.3	*39.8	2.51
Treatment	9	322	35.8	*23.2	2.33
perfusate pH	1	34.5	34.5	*22.4	5.29
adenosine	4	255	63.8	*41.4	3.01
interaction	4	42.5	10.6	*6.9	3.01
Error	63	97	1.54		
Total	79				

Table A5. ANOVA (2x5 Factorial)

Source	df	SS	Ms	Cal.F	Tab.F
Blocks	7	134	19.1	*5.62	2.51
Treatment	9	1234	137	*40.3	2.33
perfusate pH	1	292	292	*85.9	5.29
adenosine	4	864	216	*63.5	3.01
interaction	4	78	19.5	*5.74	3.01
Error	63	214	3.4		
Total	79				

Analysis of Variance (ANOVA) Tables computed to determine if linearity occurs when regressing coronary flow on perfusate adenosine concentration (Table A6); when regressing coronary flow on perfusate H^+ activity (Table A7), and when regressing coronary flow on perfusate adenosine concentration in hearts perfused first at pH 7.20 (Table A8).

Table A6. ANOVA

Source	df	SS	MS	Cal.F	Tab.F
Treatment	2	75	37.5	*20.2	3.40
Regression	1	62.6	62.6	*33.6	4.26
Remainder	1	12.4	12.4	* 6.7	4.26
Error	24	45	1.86		
Total	26				

$b = 0.0013$
$S_b^- = 0.0045$

Table A7. ANOVA

Source	df	SS	Ms	Cal.F	Tab.F
Treatment	3	56.8	18.9	*19.7	3.72
Regression	1	56.1	56.1	*58.4	5.72
Remainder	2	0.78	0.39	0.41	4.32
Error	24	23.1	0.96		
Total	27				

$b = -4.80$
$S_b^- = 0.62$

continued

Table A8. ANOVA

Source	df	SS	MS	Cal.F	Tab.F
Treatment	3	438	146	*37.0	2.95
Regression	1	337	337	*85.4	4.20
Remainder	2	101	50.6	*12.8	3.34
Error	28	110	3.94		
Total	31				

$b = 0.031$

$S_{\bar{b}} = 0.01$

Analysis of Variance (ANOVA) Tables computed from experiments in which the effects of theophylline on adenosine dilation of the coronaries was studied. In Table A9 hearts were perfused first with solution of pH 7.42. In Table A10 perfusate of pH 7.20 was first used to perfuse the coronaries.

Table A9. ANOVA (2x4 Factorial)

Source	df	SS	MS	Cal.F	Tab.F
Blocks	8	146	18.3	*6.2	2.41
Treatment	7	132	18.9	*6.4	2.51
perfusate pH	1	10	10	3.4	5.29
theophylline	3	114	38	*12.9	3.34
interaction	3	8	2.66	0.9	3.34
Error	56	165	2.95		
Total	71				

* Significance ($P < 0.05$)

Table A10. ANOVA (2x5 Factorial)

Source	df	SS	MS	Cal.F	Tab.F
Blocks	8	176	22	*5.5	2.41
Treatment	9	530	58.9	*14.7	2.33
perfusate pH	1	357	357	*84.3	5.29
theophylline	4	161	40.3	*10.1	3.01
interaction	4	12	3	0.75	3.01
Error	72	288	4.0		
Total	89				

* Significance ($P < 0.05$)

APPENDIX B

RAW DATA

Table B1. Effect of adenosine (10^{-8}-$5x10^{-7}$M) on coronary flow at perfusate pH 7.42 and 7.69. (Results, Figure 12, Table 5)

Key: C = control; M = mean, D = diastolic, S = systolic (pH 7.42)

C			10^{-8}M			$5x10^{-8}$M			10^{-7}M			$5x10^{-7}$M		
M	D	S	M	D	S	M	D	S	M	D	S	M	D	S
6.6	10.0	3.0	6.6	10.0	2.5	6.8	7.5	2.5	7.6	10.0	2.0	14.0	19.0	7.0
5.6	11.0	2.0	5.6	11.0	2.0	6.2	12.2	2.0	6.7	12.0	2.0	12.0	18.5	5.5
6.3	12.5	2.0	6.0	11.5	2.0	6.0	11.0	2.0	6.8	12.0	2.0	10.2	17.5	5.0
7.0	11.0	4.5	7.0	11.0	4.5	7.3	12.0	4.0	8.3	13.0	5.0	13.8	21.5	8.5
6.0	8.5	4.5	5.8	8.0	4.5	7.5	10.0	6.0	8.8	11.5	7.0	14.8	20.0	12.5
5.0	10.0	1.0	5.0	9.5	1.0	5.3	10.5	0.5	5.8	10.5	0.5	9.4	17.5	2.5
6.0	12.0	1.0	6.0	12.0	1.0	6.6	13.0	1.0	7.0	13.5	1.5	12.0	22.0	4.5
7.0	10.0	3.0	7.0	10.0	3.0	7.6	12.0	4.0	9.6	16.5	6.0	16.0	26.5	10.0
$\bar{X}$ 6.2	10.6	2.6	6.1	10.4	2.7	6.7	11.3	2.8	7.8	12.4	3.3	18.8	20.3	6.9

continued

Table B1--continued

(pH 7.69)

C			10^{-8}M			$5x10^{-8}$M			10^{-7}M			$5x10^{-7}$M		
M	D	S	M	D	S	M	D	S	M	D	S	M	D	S
6.0	10.0	2.5	6.2	10.0	2.5	6.4	10.0	2.5	6.6	9.5	2.5	8.7	13.5	7.0
5.4	9.5	1.5	5.4	9.5	1.5	5.7	9.5	1.5	6.0	10.0	1.5	8.7	13.5	4.5
5.2	10.5	2.0	5.3	10.5	2.0	5.5	11.5	2.0	6.0	11.5	2.5	7.7	12.5	3.0
6.4	10.0	3.0	6.3	10.0	3.0	7.0	10.0	3.0	7.0	10.0	3.0	9.6	14.0	5.0
6.0	7.5	3.5	6.0	7.5	3.5	6.8	7.5	3.5	6.7	8.0	3.5	8.8	10.0	5.0
4.5	9.5	1.0	4.5	9.5	1.0	4.8	10.0	1.0	4.8	10.0	1.0	6.0	11.5	1.5
5.6	10.5	0.5	5.6	10.5	0.5	6.2	11.0	0.5	6.0	11.0	0.5	7.4	12.5	1.0
7.2	9.5	4.0	7.2	9.5	4.0	7.5	11.0	2.5	7.3	11.0	3.0	11.2	17.5	6.5
$\bar{X}$ 5.8	9.6	2.2	5.7	9.6	2.2	6.2	10.1	2.1	6.3	10.1	2.1	8.6	13.5	4.2

Table B2. Effect of adenosine (10^{-8}-$5x10^{-7}$M) on heart rate at perfusate pH 7.42 and 7.69 (Results, Table 5) C = control

	C		10^{-8}M		$5x10^{-8}$M		10^{-7}M		$5x10^{-7}$M	
pH	7.42	7.69	7.42	7.69	7.42	7.69	7.42	7.69	7.42	7.69
	240	240	240	240	228	240	228	264	264	264
	228	240	228	240	240	240	210	240	264	240
	252	252	252	252	252	252	252	252	264	252
	264	264	264	264	264	264	264	264	264	264
	264	276	264	276	276	276	276	276	276	288
	264	252	264	252	252	252	252	252	252	264
	240	240	240	240	240	240	240	240	240	258
	252	264	264	264	240	252	240	252	240	264
$\bar{X}$	251	254	251	254	248	252	249	255	258	262

Table B3. Effect of adenosine (10^{-8}-10^{-6}M) on coronary flow at perfusate pH 7.42 and 7.26 (Results, Figure 4, Table 2) C = control flow

	C		10^{-8}M		10^{-7}M		10^{-6}M	
pH	7.42	7.36	7.42	7.36	7.42	7.36	7.42	7.36
	6.6	8.0	6.6	8.4	8.7	9.4	11.6	10.3
	7.0	8.0	7.0	8.2	7.4	9.0	10.8	10.0
	7.0	7.8	7.1	8.0	7.4	8.5	10.0	10.8
	7.2	8.2	7.6	8.2	8.2	8.8	11.8	11.0
	7.2	7.8	6.2	7.7	7.0	8.0	9.8	9.0
	5.4	6.8	5.4	6.8	6.6	7.4	10.0	10.0
	4.2	5.8	4.4	5.8	5.0	6.8	11.0	11.0
	6.4	7.6	6.6	7.8	7.6	9.4	12.0	13.0
	4.8	6.8	5.0	6.8	6.0	8.8	11.6	12.0
$\bar{X}$	6.2	7.4	6.2	7.5	7.1	8.5	10.9	10.9

continued

Table B3--continued (Results, Table 2)

Spontaneous Heart Rate

pH	C		10^{-8}M		10^{-7}M		10^{-6}M	
	7.42	7.36	7.42	7.36	7.42	7.36	7.42	7.36
	258	270	258	270	270	270	270	270
	282	276	282	276	282	276	276	276
	270	270	270	270	270	264	264	264
	288	288	288	288	288	287	288	287
	270	276	270	276	276	276	276	276
	264	288	264	288	276	288	276	288
	240	246	240	240	240	240	240	240
	246	258	246	258	258	258	258	258
	222	222	222	222	216	246	216	240
$\bar{X}$	260	266	260	265	264	267	263	267

Table B4. Effect of adenosine (10^{-8}-10^{-6}M) on coronary flow at perfusate pH 7.42 and 7.20 (Results, Figure 5, Table 2) C = control flow

	C		10^{-8}M		10^{-7}M		10^{-6}M	
pH	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20
	6.3	8.6	6.2	8.8	7.4	10.0	11.8	12.3
	6.4	7.3	6.2	7.7	6.6	8.6	8.4	10.0
	7.0	9.5	7.0	10.4	8.0	11.8	12.6	13.0
	5.2	7.6	5.6	7.4	6.0	8.8	9.0	9.8
	4.8	7.7	4.8	7.6	5.8	9.8	11.6	11.4
	5.0	7.1	5.2	7.0	6.0	10.0	13.2	14.0
	5.3	7.4	4.8	7.8	6.2	11.8	14.2	14.8
	6.2	7.4	5.8	7.6	6.8	9.8	11.2	12.4
	5.6	7.4	5.8	7.4	6.5	9.3	9.6	10.7
$\bar{X}$	5.8	7.7	5.7	8.0	6.6	9.7	11.3	12.1

continued

Table B4--continued (Results, Table 2)

	Spontaneous Heart Rate							
	C		10^{-8}M		10^{-7}M		10^{-6}M	
pH	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20
	240	238	240	234	240	240	240	240
	288	300	288	300	300	300	300	300
	246	240	246	240	246	240	246	240
	276	258	276	258	270	258	270	258
	228	234	228	234	234	234	234	234
	216	238	216	258	246	238	238	238
	318	282	276	288	288	288	294	288
	234	234	228	234	234	234	246	246
	276	276	276	276	276	276	276	276
$\bar{X}$	258	255	252	256	259	256	260	258

Table B5. Effect of adenosine (10^{-8}-$5x10^{-7}$M) on coronary flow at perfusate pH 7.42 and 6.89 (Results, Figure 6, Table 2) C = control flow

	C		10^{-8}		$5x10^{-8}$		10^{-7}		$5x10^{-7}$	
pH	7.46	6.89	7.42	6.89	7.42	6.89	7.42	6.89	7.42	6.89
	4.6	6.6	4.3	6.7	4.4	8.2	4.8	9.6	6.0	14.6
	5.0	7.4	5.4	7.8	5.2	10.4	6.0	11.6	8.8	14.2
	5.2	8.4	5.6	7.8	6.0	8.5	6.5	10.0	10.4	11.0
	5.7	7.9	5.3	7.8	5.6	9.0	5.9	10.2	9.8	14.0
	5.5	7.7	5.4	8.0	5.5	10.0	6.5	11.3	10.0	14.4
	5.4	7.3	5.4	7.0	5.3	8.6	5.8	10.3	7.0	14.0
$\bar{X}$	5.2	7.4	5.2	7.6	5.3	9.1	5.9	10.5	8.9	13.7

continued

Table B5--continued (Results, Table 2)

	Spontaneous Heart Rate									
	C		10^{-8}		$5x10^{-8}$		10^{-7}		$5x10^{-7}$	
pH	7.42	6.89	7.42	6.89	7.42	6.89	7.42	6.89	7.42	6.89
	264	276	264	276	264	264	264	264	276	276
	264	276	264	276	264	264	276	264	276	264
	264	246	264	252	252	252	252	264	276	264
	252	264	264	264	264	264	264	264	276	264
	252	264	252	264	252	264	252	252	264	252
	264	252	264	240	264	240	264	240	264	240
$\bar{X}$	260	263	262	262	260	258	262	258	272	260

Table B6. Effect of adenosine on coronary flow in hearts perfused first at pH 7.20 followed by perfusion at pH 7.42 (Results, Figure 8, Table 3)
C = control flow

	C		10^{-8}		10^{-7}		$5x10^{-7}$		10^{-6}	
pH	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42
	6.4	5.3	6.6	5.2	11.5	6.0	16.2	11.5	17.2	13.8
	5.4	4.4	5.6	4.0	12.2	4.7	16.6	9.6	17.8	13.0
	5.2	4.3	5.2	4.0	8.0	4.2	11.6	5.6	17.5	8.8
	7.8	6.0	8.0	6.0	10.4	6.6	13.8	8.0	15.0	10.6
	6.6	5.8	6.6	5.6	11.4	5.8	17.5	7.5	18.4	10.8
	8.2	6.6	8.4	6.8	14.0	7.4	18.0	12.2	19.6	15.7
	8.4	6.0	8.5	5.8	13.2	6.2	14.3	10.8	14.3	10.0
	8.8	6.8	8.9	6.8	13.0	7.4	16.8	10.6	18.0	14.4
$\bar{X}$	7.1	5.7	7.2	5.5	11.7	6.0	15.6	9.5	16.6	18.4

continued

Table B6--continued (Results, Table 3)

	Heart Rate									
	C		10^{-8}		10^{-7}		$5x10^{-7}$		10^{-6}	
pH	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42
	240	252	240	252	252	252	264	252	276	276
	240	252	240	252	268	240	264	252	276	276
	228	228	228	228	216	228	216	228	216	228
	264	264	264	264	264	264	276	264	276	264
	264	252	264	252	264	264	264	264	264	276
	264	264	264	264	264	276	276	264	276	276
	246	264	240	268	252	264	264	264	264	276
	240	252	240	252	240	264	252	264	252	276
$\bar{X}$	248	254	248	254	252	257	255	257	263	269

Table B7. Effect of adenosine ($8x10^{-7}M$) on coronary flow at pH 7.20 for 30 min. (Results, Figures 10, 11, Table 4)

	Time (minutes)						
	0	5	10	15	20	25	30
	7.2	15.4	16.0	16.7	16.8	16.8	16.8
	8.0	18.8	18.9	19.4	19.4	19.4	19.4
	9.5	17.8	18.0	17.7	17.4	17.8	17.8
	9.6	17.0	17.6	17.2	17.0	16.2	16.4
	8.5	13.6	16.0	12.6	12.0	11.2	11.2
$\bar{X}$	8.6	16.4	16.9	16.7	16.5	16.3	16.3

continued

Table B7--continued (Results, Table 4)

	Heart Rate						
	Time (minutes)						
	0	5	10	15	20	25	20
	228	228	228	228	228	228	228
	204	216	216	216	216	216	216
	228	240	236	236	230	230	230
	252	252	252	264	264	264	264
	252	246	246	240	252	252	252
$\bar{X}$	233	236	236	237	238	238	238

Table B8. Effects of pH on flow (F) and heart rate (HR), and on the flow response to adenosine ($5x10^{-7}M$) = A (Results, Figure 13, Table 6)

pH	F	A	HR	pH	F	A	HR
6.88	9.4	13.6	228	7.22	8.2	10.3	388
6.90	10.2	18.4	264	7.18	7.2	12.8	252
6.84	8.6	17.0	216	7.15	6.6	13.0	238
6.91	11.2	20.4	240	7.23	8.2	15.0	240
6.88	7.5	23.0	252	7.20	6.7	13.8	252
6.90	7.8	18.0	252	7.21	6.5	12.4	252
6.89	9.2	18.5	276	7.19	7.3	13.8	276
pH	F	A	HR	pH	F	A	HR
7.43	7.4	8.5	288	7.66	6.2	7.6	288
7.97	5.2	7.5	240	7.72	4.2	5.4	240
7.46	6.5	7.2	238	7.66	4.0	5.2	240
7.48	7.0	9.4	240	7.67	6.2	8.6	252
7.40	5.4	9.8	252	7.74	5.8	7.4	252
7.40	5.8	8.2	252	9.69	5.0	6.6	252
7.42	6.8	9.4	276	7.70	6.0	7.8	276

Table B9. Effect of theophylline on coronary flow at perfusate pH 7.42 followed by pH 7.20 (Results, Figure 15, Table 7) C = control flow

	C		10^{-6}		10^{-5}		$5x10^{-5}$		10^{-4}	
pH	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20
	3.8	4.4	3.8	4.7	3.8	4.7	4.0	4.8	4.0	5.1
	4.8	5.4	4.8	5.4	9.4	5.6	4.4	5.8	5.8	6.0
	5.0	5.6	5.2	5.4	4.6	4.9	5.2	4.7	5.0	5.4
	5.5	6.3	5.5	6.3	5.4	5.8	5.8	6.1	5.5	6.5
	5.3	5.6	5.3	5.6	5.0	5.8	5.0	5.8	5.0	5.8
	5.3	6.4	4.9	6.4	5.0	6.4	5.0	6.3	5.1	6.6
$\bar{X}$	4.9	5.6	4.9	5.6	4.7	5.5	4.9	5.6	5.0	5.9

continued

Table B9--continued

Spontaneous Heart Rate

pH	C		10^{-6}		10^{-5}		$5x10^{-5}$		10^{-4}	
	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20
	228	216	228	228	228	228	228	228	228	228
	240	252	240	264	252	264	252	252	264	264
	264	264	252	264	252	264	264	252	252	264
	228	216	216	228	228	216	228	228	240	228
	276	252	268	240	252	252	252	252	252	252
	258	288	288	288	276	288	276	288	288	288
$\bar{X}$	249	248	243	252	248	252	250	250	254	254

Table B10. Effect of theophylline ($5x10^{-4}$ and 10^{-3}M) on coronary flow and spontaneous heart rate at perfusate pH 7.42. (Results, Figure 17, Table 7) C = control

Flow			Rate		
C	$5x10^{-4}$	10^{-3}	C	$5x10^{-4}$	10^{-3}
5.1	6.3	8.0	252	264	312
6.0	8.0	11.4	252	276	324
5.8	6.8	9.6	216	228	312
7.6	9.4	12.6	312	342	360
7.0	8.3	10.8	264	300	324
6.0	5.2	9.4	288	324	372
$\bar{X}$ 6.1	7.3	10.3	264	289	334

Table B11. Effect of theophylline on coronary flow produced by adenosine ($8x10^{-7}M$) at perfusate pH 7.42 followed by pH 7.20 (Results, Figure 18, Table 8)

	C		Adenosine		$10^{-6}M$ Theo.		$10^{-5}M$ Theo.		$10^{-4}M$ Theo.	
pH	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20
	6.2	7.6	12.4	12.2	12.2	12.2	11.6	11.8	7.8	10.0
	6.2	7.8	10.0	10.2	9.6	10.3	8.4	10.2	7.2	9.0
	9.0	10.2	13.6	14.2	13.8	14.4	13.4	14.0	10.8	12.4
	8.8	10.0	14.4	15.0	13.8	14.8	13.6	14.8	10.8	12.6
	7.2	7.5	10.0	11.0	10.5	11.2	9.2	10.8	7.2	9.2
	6.8	8.6	11.0	12.8	10.6	12.6	10.0	12.0	8.6	10.6
	5.0	5.8	11.8	11.2	11.8	11.0	10.6	10.2	7.2	8.2
	7.0	6.8	11.8	11.0	11.8	11.0	10.8	9.6	6.8	7.2
	5.4	6.2	12.0	12.2	12.2	12.2	9.8	10.8	7.0	8.8
$\bar{X}$	6.8	7.8	12.0	12.2	11.8	12.2	10.8	11.6	8.2	9.8

continued

Table B11--continued

	Heart Rate									
	C		Adenosine		10^{-6}M Theo.		10^{-5}M Theo.		10^{-4}M Theo.	
pH	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20
	276	288	276	288	288	294	300	294	300	294
	228	252	228	252	252	252	258	252	270	252
	288	282	288	282	288	288	282	288	282	288
	264	264	270	270	270	270	264	264	264	264
	276	264	276	270	276	270	276	276	276	276
	264	258	264	258	258	264	258	264	258	264
	264	264	264	264	258	264	258	282	258	282
	246	276	246	276	246	276	276	276	288	276
	234	240	228	240	252	246	258	240	252	240
$\bar{X}$	260	265	260	267	265	269	270	271	272	271

Table B12. Effect of theophylline on coronary flow produced by adenosine ($8x10^{-7}M$) in hearts perfused first at pH 7.20 followed by perfusion at pH 7.42. (Results, Figure 19, Table 9)

	C		Adenosine		$10^{-6}M$ Theo.		$10^{-5}M$ Theo.		$5x10^{-5}M$ Theo.		$10^{-4}M$ Theo.	
pH	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42	7.20	7.42
	8.0	7.6	17.0	12.2	16.0	12.2	14.0	11.2	13.0	9.6	11.8	9.2
	6.8	6.2	11.6	8.6	11.8	8.6	11.4	7.0	11.1	6.8	9.0	6.4
	6.2	4.2	13.6	10.6	13.6	10.6	13.2	9.8	12.3	8.2	10.4	7.8
	7.6	6.0	13.6	9.4	13.6	9.6	12.0	9.2	11.6	7.8	10.8	7.6
	6.4	5.6	13.0	10.2	13.0	10.0	12.0	9.4	11.2	8.0	10.4	7.0
	6.0	5.4	13.0	9.8	13.0	9.8	12.4	8.8	10.0	7.0	8.2	6.8
	5.4	3.8	12.4	7.6	12.4	7.6	11.4	6.2	8.4	5.0	7.2	4.6
	7.4	6.4	18.2	11.0	18.2	11.2	13.8	10.6	11.0	10.4	11.6	10.4
	5.5	3.8	15.6	6.2	18.4	6.2	14.2	5.0	11.2	4.6	10.0	4.8
$\bar{X}$	6.6	5.4	14.2	9.5	14.1	9.5	12.8	8.6	11.1	7.6	9.9	7.1

Table B13. Effect of concurrent theophylline ($5x10^{-5}M$), ouabain ($1.4x10^{-7}M$) and alkalosis (perfusate pH 769) on reactive dilation. (Results, Figure 21, Table 10)

C = absence of test agents, E = presence of test agents

Peak Flow (ml/min)			VCF_{20}			T_{50}		
C	E	C	C	E	C	C	E	C
15.0	14.0	15.0	3.6	3.3	3.5	12.5	12.3	14.5
14.0	13.5	13.0	3.6	8.1	3.1	16.0	14.2	14.5
17.5	15.0	17.0	3.7	3.2	3.3	14.0	12.4	11.5
13.5	13.0	13.0	3.1	2.4	2.2	13.5	10.5	10.0
14.0	13.0	15.0	3.5	3.2	3.3	13.5	14.0	11.0
16.0	16.5	14.0	3.9	3.8	3.6	13.5	12.0	9.2
18.0	17.0	17.5	4.6	4.0	4.3	15.0	12.2	13.5
17.5	15.5	15.0	3.6	2.8	2.8	13.0	10.5	11.0
$\bar{X}$ 15.7	14.7	14.9	3.7	3.2	3.3	13.9	12.3	11.9

Table B14. Effect of concurrent theophylline ($5x10^{-8}M$), ouabain ($1.4x10^{-7}M$) and alkalosis (perfusate pH 7.69) on hypoxic coronary flow.

C = control, A = absence of test agents,
P = presence of test agents

Coronary Flow												
Mean				Systolic				Diastolic			
C	A	C	P	C	A	C	P	C	A	C	P
6.6	12.2	6.6	12.6	0.5	4.0	0.5	5.5	12.0	22.0	12.0	21.0
4.4	14.2	4.0	14.2	5.0	10.0	3.0	10.5	6.0	20.5	6.0	20.0
7.4	13.0	7.4	12.8	1.5	5.5	2.5	7.0	15.5	24.0	18.0	21.0
6.6	14.2	6.6	14.0	2.0	3.5	1.5	2.5	13.0	25.0	11.0	21.0
4.0	11.2	4.0	18.2	3.0	7.5	2.0	6.5	5.5	17.5	5.0	18.0
6.2	17.2	6.3	16.4	1.0	12.5	1.0	11.0	11.5	23.0	11.0	21.0
7.6	16.6	7.2	16.0	4.0	9.5	3.5	10.0	11.5	25.5	10.5	23.0
$\bar{X}$ 6.1	14.1	6.0	14.0	1.0	7.5	1.0	7.6	10.7	27.5	10.5	21.5

Table B15. Effect of concurrent theophylline ($5x10^{-5}M$), ouabain ($1.4x10^{-7}M$), and alkalosis (perfusate pH 7.62) on autoregulation in the coronary vasculature. (Results, Figure 22, Table 11)

A = absence of test agents P = presence of test agents

Control Presence (65 cm H_2O)				Elevated Pressure (95 cm H_2O)				Lowered Pressure (35 cm H_2O)			
Stable Flow		Resistance		Stable Flow		Resistance		Stable Flow		Resistance	
A	P	A	P	A	P	A	P	A	P	A	P
4.8	5.0	13.5	13.0	7.0	6.8	13.6	14.0	4.2	3.2	8.3	10.9
5.2	12.5	12.5	11.8	6.4	5.8	14.8	16.4	4.0	3.0	8.8	11.7
6.8	6.5	9.6	10.0	8.6	7.4	11.0	12.8	5.0	4.6	7.0	7.6
6.3	6.1	10.6	10.1	8.5	7.3	11.5	13.3	4.5	3.7	8.0	9.8
6.3	5.6	10.3	11.6	7.4	6.7	12.8	41.2	4.0	3.2	8.8	10.9
7.4	7.0	8.8	9.3	9.0	8.4	10.6	11.3	4.5	4.0	7.8	8.8
8.3	8.4	7.8	7.7	10.4	9.0	9.1	10.6	4.5	4.0	7.8	8.8
5.5	4.6	11.8	14.1	11.0	6.8	9.6	14.0	4.6	7.6	7.6	9.7
$\bar{X}$ 6.3	6.1	10.6	9.8	8.5	7.3	11.5	13.3	4.4	3.7	8.0	9.5