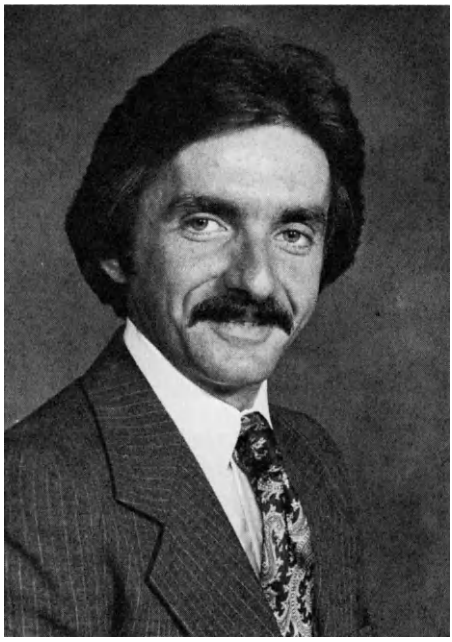

ERWIN MARGULIES
1946–1980

Myocardial Infarction and Cardiac Death

Edited by
Erwin Margulies
Pharmaceuticals Division
CIBA-Geigy Corporation
Summit, New Jersey

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for Libby, Gayle, and Michael

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In Memoriam

This volume is dedicated to the memory of Erwin Margulies who had engaged the authors for this work prior to his untimely death. As editor-in-chief of this series I have sought to consummate, as best possible under the circumstances, what he had initiated so that it would serve as a fitting tribute to a person who was taken from us just as he was beginning to fulfill his great potential.

Dr. Margulies was born on June 1, 1946, and died in an automobile accident on April 17, 1980. In his 34 years he had accomplished a great deal. After earning the Ph.D. in physiology from Temple University School of Medicine in 1973, he joined the Pharmaceuticals Division of CIBA-Geigy as a senior clinical associate. At the time I was executive vice president and director of research at CIBA-Geigy and it was quite evident to my colleagues and myself that we had engaged not only a scientific talent but a person with notable managerial abilities. His first task was responsibility for thromboembolic clinical trials followed by assignment as principal medical monitor of the Anturane Reinfarction Trial (ART) for North America. In 1978 Dr. Margulies was promoted to senior director, Methodology and Long-Term Intervention Trials for CIBA-Geigy. It was at this time that I approached Dr. Margulies to prepare a monograph on myocardial infarction and cardiac death for the *Medicinal Chemistry* Series. His immediate positive response was followed with a series of communications to me concerning the authors he had committed to the task as well as the scope of the work. This opus represents an

unfinished volume because Dr. Margulies was to submit a chapter. I wish to express my appreciation and gratitude to all the authors who have participated in bringing this volume to fruition. I personally am pleased to dedicate this book to the memory of Dr. Margulies as a fine scientist, a loyal colleague, and a good friend.

George deStevens
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Preface

The greatest cause of morbidity and mortality to Western mankind is myocardial infarction and cardiac arrest. This became more pronounced in the 20 to 25 years following World War II for a variety of reasons including diet, life style, inherent personality characteristics and cultural influences. Associated with these factors has been the underlying principle that cholesterol and other lipids were predominant culprits leading to atherosclerosis and eventual coronary accidents. Consequently, a leading approach in a number of laboratories was the synthesis and biological evaluations of hypolipidemic agents. This course of action was pursued for a number of years leading to the development of substances such as clofibrate and nicotinic acid for the treatment of hyperlipidemia. Although these drugs indeed lowered cholesterol levels, several epidemiological studies revealed that a corresponding decrease in myocardial infarction did not occur. In addition, the adverse reactions caused by these drugs gave additional pause as to the benefit derived versus the serious risk factors associated with long term therapy.

Thus, another approach to this difficult medical problem was necessary. Within the past 15 years the focus of attention of researchers has been on the study and understanding of the biochemical events associated with this disease at the molecular level. In this regard, the elucidation of the arachidonic acid cascade leading to a detailed knowledge of the role of prostacyclin and thromboxane in controlling platelet formation has been a milestone in this research. Moreover, the advent of β-adrenergic blockers and their influence in overcoming the positive inotropic effect associated with catecholamine-β-receptor interaction has also been of major importance. Both of these approaches to the

treatment of myocardial infarction are covered in this volume. In fact, major efforts are underway in several research based pharmaceutical laboratories to develop thromboxane inhibitors and also long-acting orally active prostacyclin derivatives with greater specificity (i.e., free from the cardiovascular effects associated with prostacyclin). The exciting preclinical and clinical research forthcoming from these laboratories in the next few years will certainly bear watching and will have a significant effect on the prevention and treatment of myocardial infarction.

George deStevens
Editor-in-Chief
Medicinal Chemistry Series

1 Role of the Microvasculature in Myocardial Infarction

MARY P. WIEDEMAN

I. THE MICROVASCULATURE OF THE MYOCARDIUM

A. Introduction

Of prime importance in understanding the effect on the microcirculation of alterations in blood flow as would occur in myocardial infarction is knowledge of the anatomical and functional characteristics of the blood vessels that constitute this portion of the vascular system.

In a broad sense, the terminal vessels of the arterial distribution which are considered to mark the beginning of the microcirculation are the small arterioles that are parent vessels for terminal arterioles. Terminal arterioles are so designated because they give rise only to capil-

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laries, do not anastomose with any other vessels, nor do they have any termination other than a capillary network. Postcapillary venules are formed by the confluence of capillaries and they, in turn, join to form venules. These venules mark the end of the vessels that are considered to belong to the microcirculation. The size of the vessels does not qualify them as part of the microvasculature, because obviously size will vary among species and tissues. The designation is based on the anatomical position of the vessels in the vascular tree. Admittedly, it is difficult to agree on the architecture of a "typical" bed; however, Fig. 1 is acceptable to indicate the relationships of various components in a schematic fashion.

Control of blood flow through the exchange vessels of the circulatory system is primarily through local influences. Sympathetic vasoconstrictor nerves are not found at the level of the terminal arterioles, so signals emanating from the vasomotor center can only exert an effect upstream. The activity of the vascular smooth muscle that invests the microvessels is regulated by two means. One is a myogenic response, evoked by variations in intraluminal or transmural pressure that alter tension in the smooth muscle cells. The second is a metabolic control, which depends on the response of vascular smooth muscle to metabolites that accumulate locally, most of which have a vasodilatory effect.

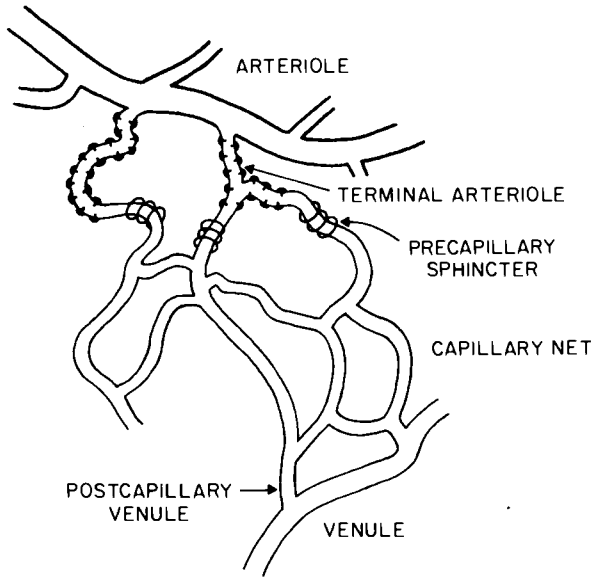

Fig. 1. A schematic diagram of a capillary network that shows the terminology for vessels of the microcirculation [Wiedeman et al. (1981)].

The termination of the vascular smooth muscle investment of the arterial distribution is marked by the precapillary sphincter which exhibits spontaneous, rhythmical contractile activity and whose contracted and relaxed phases are modified both by pressure and metabolites. Regulation of blood flow into the capillary exchange vessels by this combination of influences permits the proper distribution of blood to meet the needs of the tissue it serves and assures an appropriate supply of blood in spite of alterations in peripheral resistance and cardiac output.

With this general descriptive material as a background, consideration must now be given to the microvessels of the myocardium.

B. Methods of Preparation for Visualization of the Microcirculation

Microscopic studies of vessels in the myocardium of the beating heart are extremely difficult because of the technical problems associated with making observations of moving tissue at high magnification. In addition, the high density of the capillaries in this tissue compounds the problem. The *in vivo* studies are necessary to determine parameters such as velocity of flow, variations in vascular diameters, and evidence of regulation and control of flow. Some accuracy regarding the architecture can be obtained from fixed sections of heart muscle, but the question of technical artifacts always arises.

One of the first *in vivo* studies was designed by Martini and Honig (1969) who used cinematography to capture the blood vessels in the beating heart. Capillaries were observed by epillumination at a depth of about 20 μm from the epicardial surface. With selected eyepieces and an objective with a long working distance, the heart could move with respiration and systole without hitting the objective and the magnification used was sufficient to observe individual erythrocytes. A suitable field was found and the microscope was focused so that the bed was clearly visible during the brief cessation of respiratory movement that occurred at the end of each expiration. For each animal (rat), approximately 100–150 ft of 16-mm movie film were exposed to provide 30–50 frames that were in focus. These focused frames were used for the study to measure intercapillary distances in the beating heart.

Tillich *et al.* (1971) introduced a method to visualize cat hearts by introducing a hollow rod into the atrium through which a lucite rod was advanced to supply the light source for transillumination. The

entire preparation, the animal, light pipe, pulsed light source, and pressure gauges moved as a unit with respect to the optical train. A 16-mm camera photographed the field at 24 fps. Red blood cell velocity and capillary diameters were measured in this preparation.

A further technical advance was made by Tillmanns *et al.* (1974) who transilluminated animal hearts with a 20-gauge light transmitting needle which was placed underneath the myocardium. A system was designed to maintain the proper focal distance between the moving heart and the microscope objective. The objective of the microscope moved in unison with the cardiac surface by means of a special device called a "floating focus keeper" (see Fig. 2).

Nellis *et al.* (1981) have most recently described a method for viewing microvessels in the beating heart called the free-motion technique which can be used to gain information concerning the normal physiology of coronary circulation and permits measurements of changes in vascular pressure that may occur in an ischemic myocardium. Nellis *et al.* (1981) had previously used a technique in which a portion of a ventricle was fixed and motion of the beating heart was centered around the fixed point. This caused unwanted external forces to be exerted on the myocardium. The free-motion technique minimizes these external effects. A small portion of the right ventricle of a beating rabbit heart can be observed using a stroboscopic light source that is synchronized with the cardiac cycle. The microvessels of the coronary circulation can be visualized in this way at the same time during each

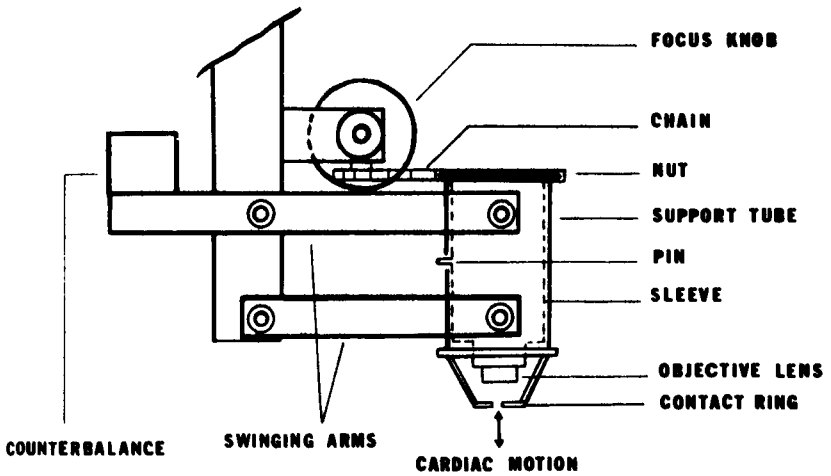

Fig. 2. Diagram of the floating focus keeper, which facilitates visualization of microvessels in the beating heart [Tillmanns *et al.* (1974)].

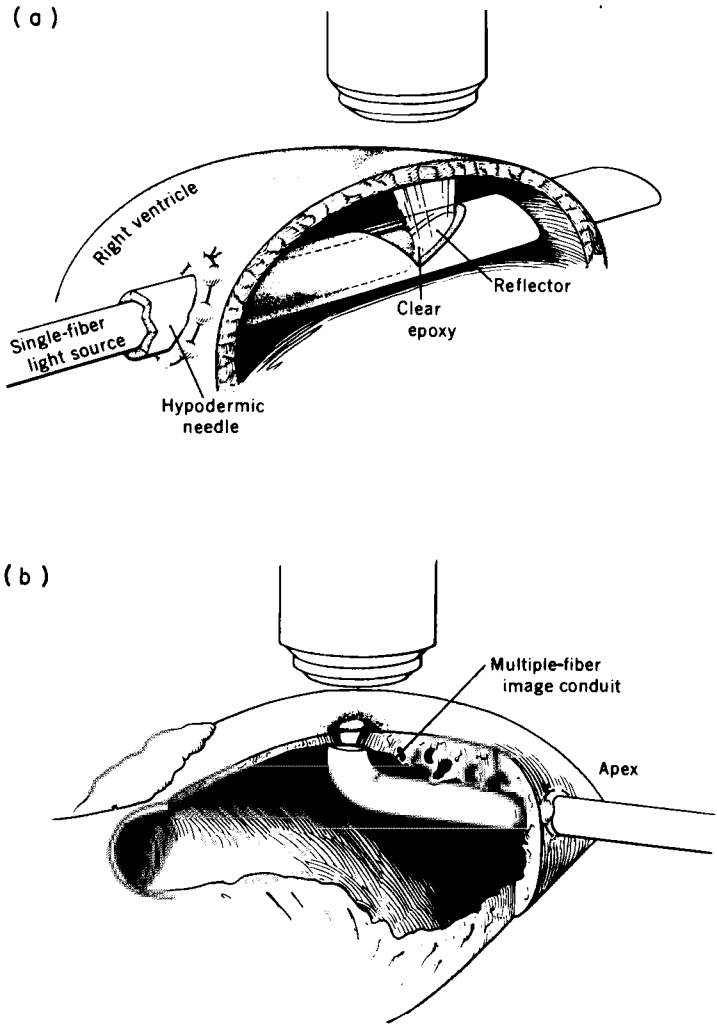

Fig. 3. A diagram of the (a) fixed-position and (b) free-motion method for *in vivo* microscopic observation of vessels in a beating heart [Nellis *et al.* (1981)].

beat and to the observer the beating heart appears to be stationary. This stopped action can be done at any phase of the cardiac cycle and by varying the pulsed illumination from the stroboscopic light source it is also possible to see the heart as if it was beating in slow motion. Pressure and diameter measurements were made using this technique. A diagrammatic representation of both the fixed-position and free-motion techniques is shown in Fig. 3.

In vitro methods, in which the coronary vessels were injected with silicone elastomer material, were employed by Bassingthwaighte *et al.* (1974). From these preparations, this group was able to make diameter and length measurements of small vessels and capillaries from which calculations of capillary surface area and capillary density were made. Grayson *et al.* (1974) used Microfil (silicone rubber) injections to describe the morphological appearance of myocardium following acute coronary occlusion. Murakami *et al.* (1978) and Phillips *et al.* (1974) achieved excellent results using a Mercox (methyl methacrylate) corrosion preparation of cat hearts to show important anatomical features of the capillaries. The descriptive material resulting from these various preparations will be presented in the text that follows.

C. Anatomical and Functional Characteristics of Microvessels of the Myocardium

Numerous anatomical features of myocardial capillaries have been described as a result of both *in vivo* and *in vitro* studies. Capillary diameter *in vivo* was seen by Tillich and co-workers (1971) to be 5.3 μm in the cat, while Tillmanns *et al.* (1974) reported diameters in the dog heart of 4.1 μm in systole and 6.3 μm in diastole. Sobin and Tremer (1972) had found a mean diameter of around 4 μm in both rat and dog hearts. In dog hearts in which vessels were maximally dilated, Bassingthwaighte *et al.* (1974) found capillaries to have diameters of 5.6 ± 1.3 μm in fixed preparations. Henquell *et al.* (1976) used stop-motion microphotographs to measure capillary diameters in rat hearts and found a mean diameter of 4.41 μm for the entire cardiac cycle with a mean of 4 μm in systole and 5 μm in diastole. It is interesting to note that there are only small differences in capillary diameters in rat, cat, and dog hearts as determined from both the beating heart and the fixed material. A capillary network from the studies of Murakami is shown in Fig. 4.

Capillary density is extremely high in cardiac muscle, reported to be between 3100 and 3800/mm^2 by various investigators. The anatomical density in a skeletal muscle such as the extensor hallucis longus or the tenuissimus for comparison is only 1000–1347/mm^2. A silicone elastomer filled section of heart muscle prepared by Bassingthwaighte *et al.* (1974), as seen in Fig. 5, clearly depicts the very dense arrangement of arteriolar and capillary vessels. The ratio of blood vessels to the muscle

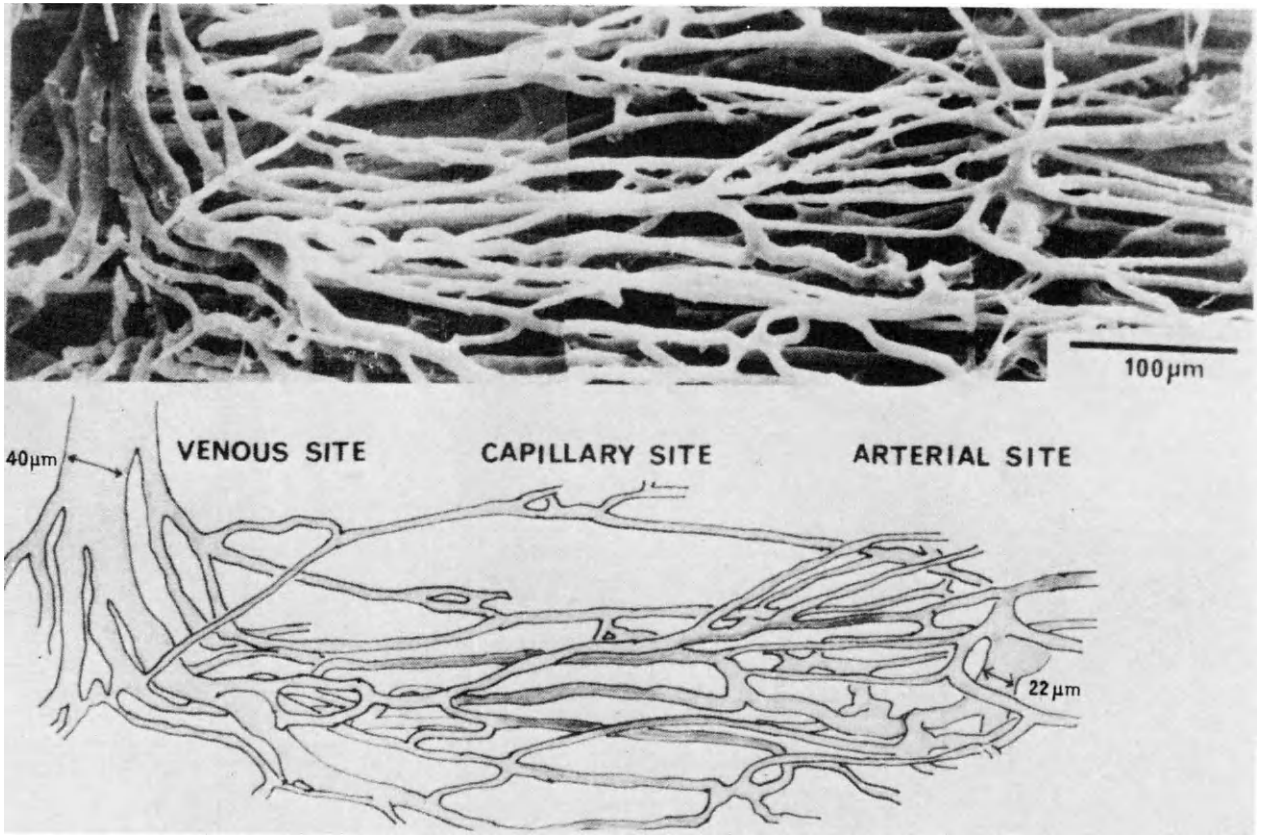

Fig. 4. A Mercox cast of a capillary bed in the myocardium of a dog [Shozawa *et al.* (1981)].

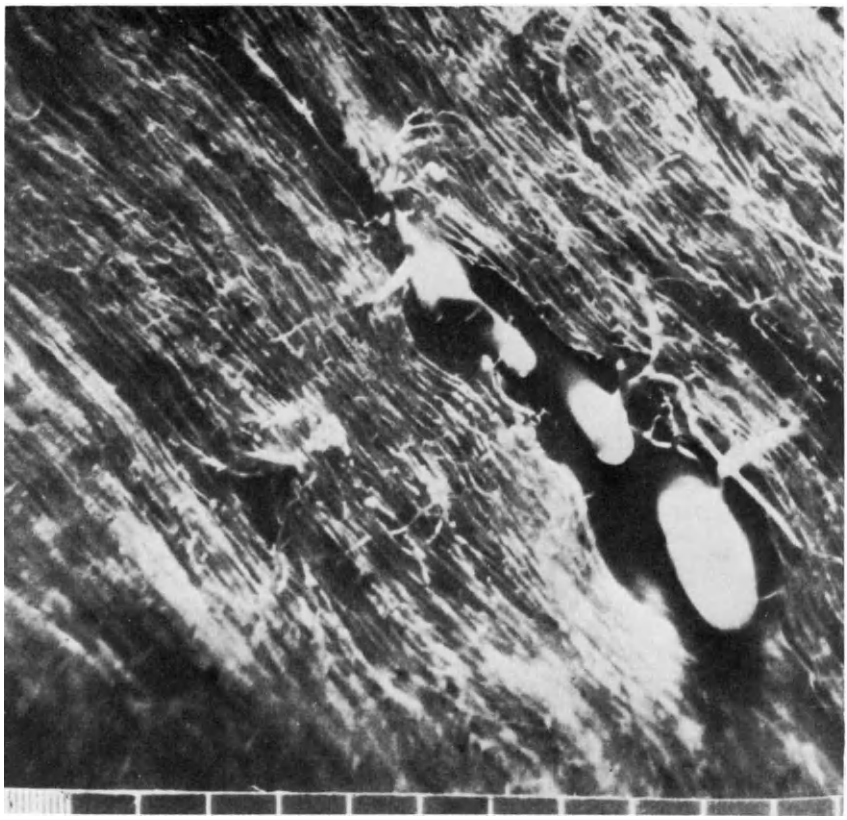

Fig. 5. Dense capillary network in the subepicardium of the dog heart [Bassingthwaighte *et al.* (1974)].

fibers, however, is close to 1.0. Rakusan *et al.* (1980), using fixed tissues from rat heart, found the number of muscle fibers to be 3200/mm^2 and capillaries were approximately 3500/mm^2. Diffusion distance was calculated to be 8.4 μm, which is thought to be less than one-half the diffusion distance for skeletal muscle. Intercapillary distances reported by Bassingthwaighte *et al.* (1974) averaged 17.4 μm; however, Martini and Honig (1969) found intercapillary distances of 18.0 to 21.4 μm with a mean of 19.5 μm. The diffusion distance is considered to be one-half these values.

The length of capillaries in cardiac muscle is very long relative to other capillary lengths that have been measured, with the exception of capillaries in the intestinal muscle layers. The capillaries in myocardium that lie parallel to the muscle fibers have been reported by

Bassingthwaighte to have functional lengths of 500 to 1000 μm in the dog heart. The vascular pattern formed by the capillaries is the same as that seen in most skeletal muscle in that numerous cross bridges connect the longitudinally oriented capillaries. The connections are described as short, perpendicular anastomoses, and they give a ladderlike appearance to the capillary network. A diagram of this familiar skeletal muscle pattern as seen in the tenuissimus muscle is shown in Fig. 6.

The presence of precapillary sphincters has been noted by several investigators, both on a functional and an anatomical basis. Observations by Feldstein et *al.* (1978) on rat hearts resulted in the conclusion that the site for control of blood flow to capillaries in the heart occurs distal to the arterioles and must be then at the precapillary sphincter. The smallest coronary arterioles supply several capillaries. Complete closure of coronary arterioles does not occur in normal hearts, and arterioles and precapillary sphincters seem to function independently of one another. Phillips and co-workers (1979) identified a narrowing at the origin of an arteriolar branch which they believed to be a precapillary sphincterlike structure (see Fig. 7). No vessels resembling true arteriovenous anastomoses have been described in either the *in vivo* or the *in vitro* studies.

The development of the techniques that permit *in vivo* microscopic observation of capillary flow has made it possible to measure the velocity of red blood cells in these small vessels in the beating heart. Tillich et *al.* (1971) transilluminated the left atrium of the cat heart and found that the red cell velocity was 112 μm/sec in capillaries when the heart was beating 160 times a minute. Tillmanns et *al.* (1974) found red cell velocity in the dog heart to be 3150 μm/sec in capillaries during systole, dropping to 1428 μm/sec in diastole, while arterioles had red cell velocities of 1168 μm/sec in systole increasing to 3391 μm/sec in diastole. In discussing the finding that arteriolar flow is maximal in diastole and capillary flow increases during systole, the investigators suggest that the cause must lie in the interrelationships between systemic blood pressure, intramural pressure, vascular cross-sectional area, and capacity. Ventricular contraction could effectively reduce coronary inflow by producing a high intramural pressure and reduce blood pressure in coronary arterioles and arteries. This would cause a decrease in red cell velocity in the arterioles. Capillaries are compressed at the same time and the blood they contain is pushed toward the venular end of the network which increases capillary red cell velocity. At the end of systole, capillary red cell velocity may be decreased because of the depletion of capillary blood volume, although there is no evidence at present to support this idea. Nellis et *al.* (1981)

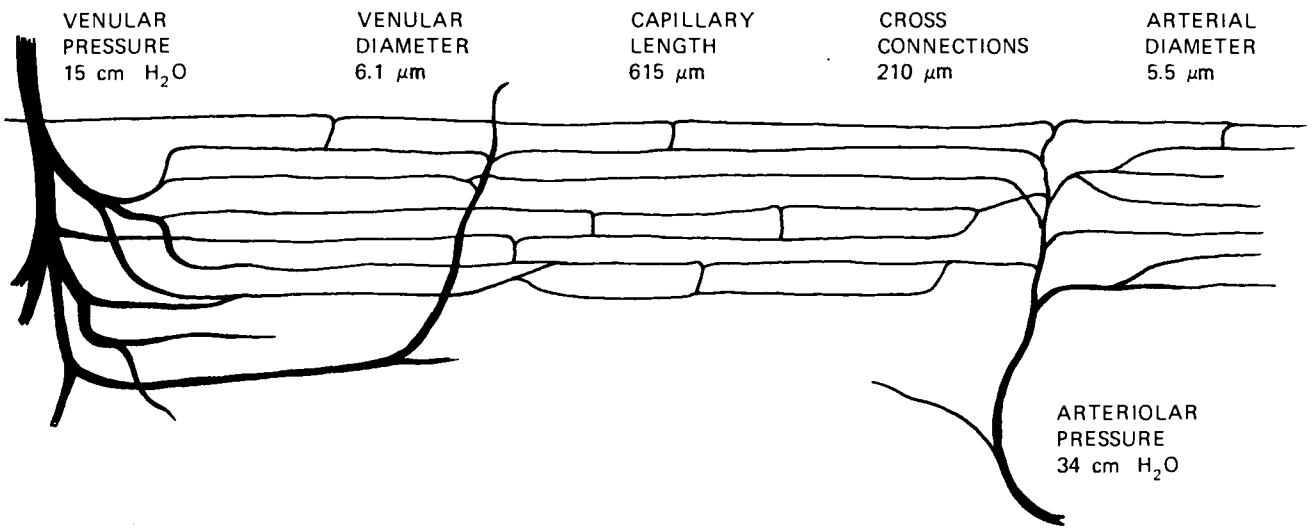

Fig. 6. A capillary bed in skeletal muscle (tenuissimus) showing the arrangement typical for skeletal muscle: Capillary density, 1300/mm^2; distance between capillaries, 34 μm; capillary surface area, 244 cm^2/cm^3 muscle. Microocclusion pressure data: arterial end, 32 cm H_2O; venular end, 22 cm H_2O. Capillary filtration coefficient, 0.001 μm^3/μm^2 sec cm H_2O difference; red cell velocity, 700 μm/sec [Smaje *et al.* (1970)].

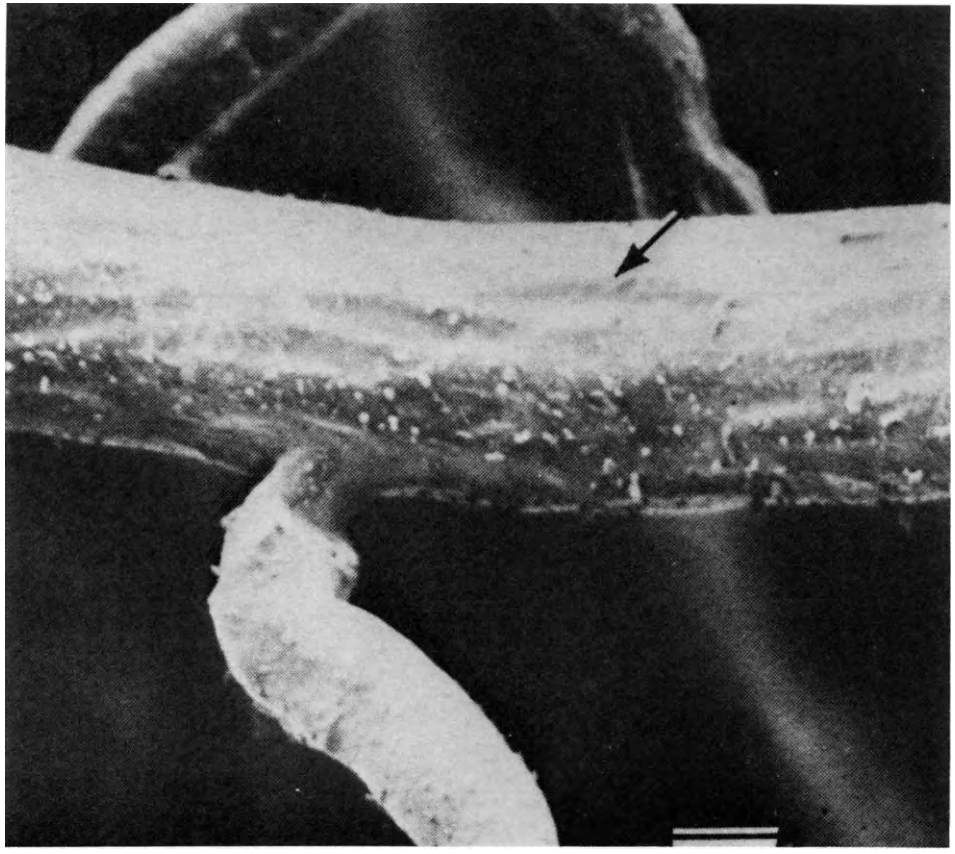

Fig. 7. A constriction of the origin of an arteriolar branch from its parent vessel resembling a precapillary sphincter site [Phillips *et al.* (1970)].

were able to measure diameters of veins and their pressure. They reported that small veins with a mean diameter of 101.5 μm had a mean pressure of 6.0 mm Hg.

It seems apparent that continuation of technical advances will foster the accumulation of more information from the beating hearts so that the functional aspects of coronary microvessels will keep pace with the descriptive material of the anatomy of the vessels and their vascular pattern, much of which has been obtained from fixed material.

II. MICROCIRCULATION IN MYOCARDIAL INFARCTION

Although there is a wealth of material describing the alterations in coronary blood flow immediately and subsequently following myocardial infarction, there are relatively few investigations that are directed to the anatomical or functional changes in the microvasculature of the beating heart of the living animal. It may be assumed that technical difficulties involved in direct microscopic observations of this constantly contracting muscle are responsible in part. Obviously, occlusion of a coronary arterial vessel will block the source of blood supply to the distally located exchange vessels that support the life of myocardial cells. What has not yet been adequately described is how the terminal vascular beds respond to this deprivation. Consequences of "low flow" or "no flow" states, such as the appearance of a collateral circulation, the release of enzymes, changes in electrolytes, and compensatory changes in the contractile activity of the myocardium, are known, but to what extent the microvessels can enter into protection against further damage to the muscle or to its maintenance during repair or restoration of flow is not yet known.

A review of several *in vitro* and *ex vivo* studies, emphasizing the effect on the microcirculation, follows.

A study in 1971 by Flohr *et al.* utilized a method to visualize the distribution of regional flow in the heart by autoradiography to assess the effects of acute coronary occlusion on the distribution of blood in the myocardium of dogs. It was demonstrated, as shown in Fig. 8, that the injected radioactive particles were not distributed homogeneously in normal hemodynamic conditions. It was not seen, however, that flow was relatively low in the subendocardial layers as had been suggested by others. After ligation of the anterior descending coronary

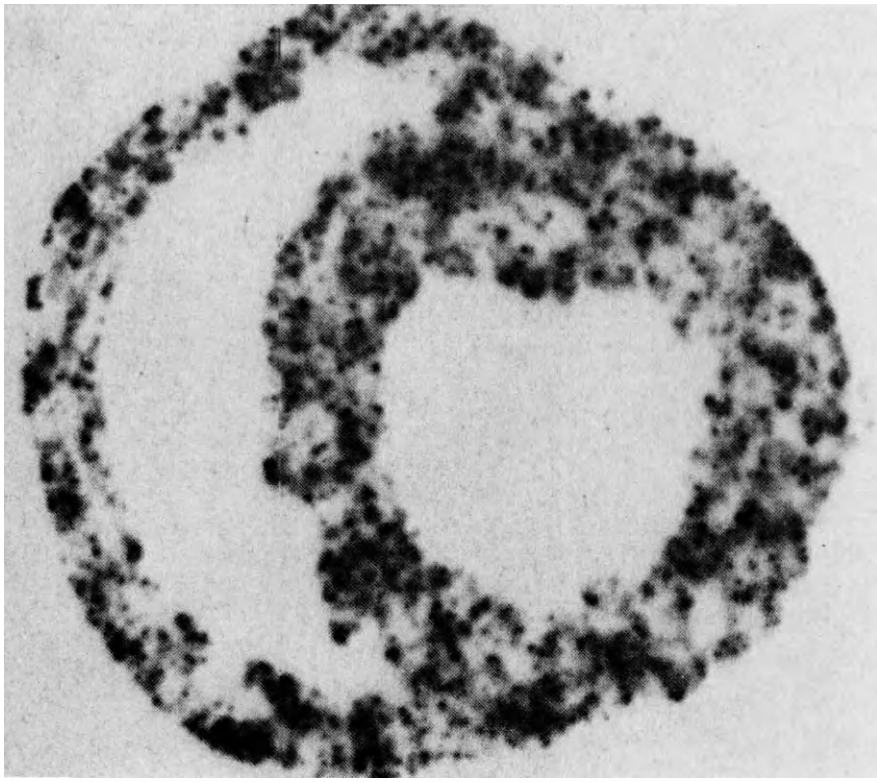

Fig. 8. Distribution of radioactive microspheres in the myocardium under normal conditions [Flohr *et al.* (1981)].

artery, ischemic zones showed a marked reduction of uptake of the radioactive particles (see Fig. 9). The reduction of uptake was most marked in the inner layers of the left ventricular wall, indicating a different auxillary circulation pathway for various areas of the heart muscle.

An *in vitro* study by Grayson and co-workers (1974) described impedance to filling of myocardial capillaries with injected silicone rubber after ligation of the anterior descending coronary artery. The injected Microfil failed to enter capillary vessels of the ischemic area resulting in a black background, which clearly indicated the region affected by the coronary occlusion (see Fig. 10). Arterial and venous vessels were still visible in the damaged area. The veins filled in a retrograde manner, not by the Microfil entering from the capillary network. The extent of the infarction varied from animal to animal, de-

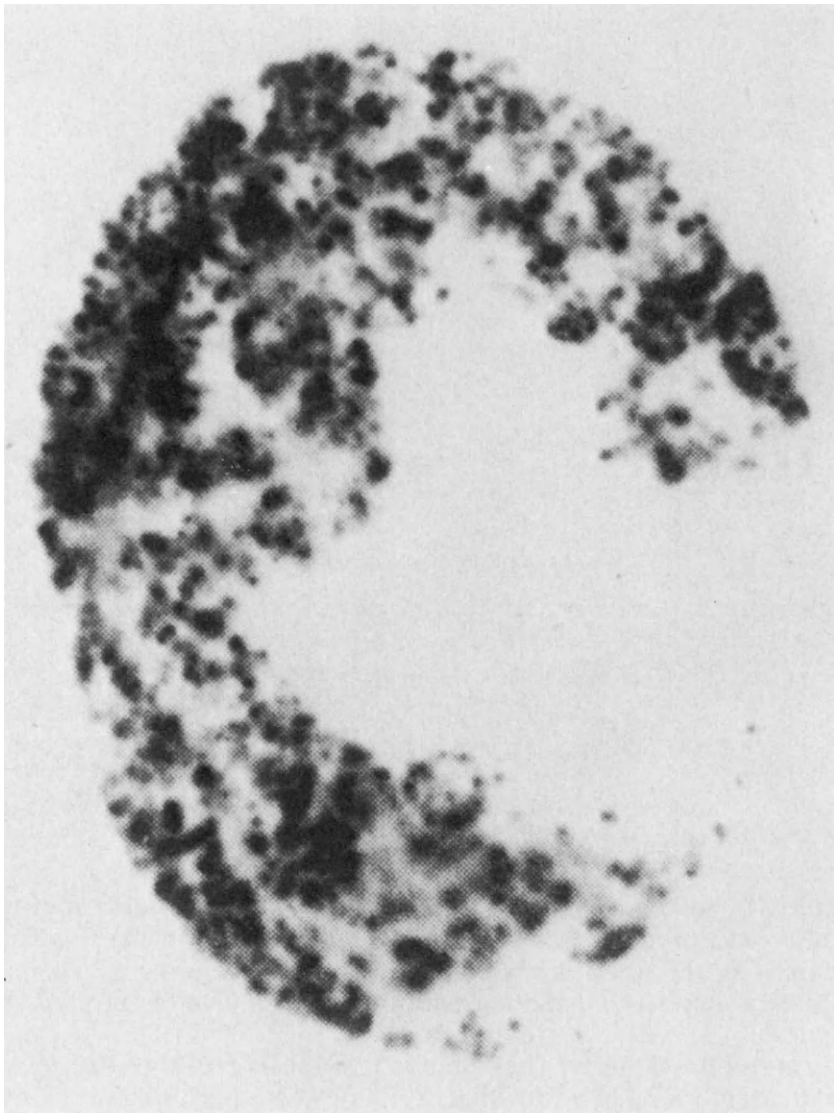

Fig. 9. Distribution of radioactive microspheres after ligation of the descending coronary artery [Flohr *et al.* (1970)].

pending on the vascularity of the preparation. Even in small areas of infarction, Microfil did not enter the capillaries, while the larger vessels continued to be filled retrogradely (see Fig. 11). To determine if the high viscosity of Microfil was responsible for the empty capillaries, the

experiments were repeated using trypan blue. Absence of capillary filling was again seen, very prominently on the subendocardial surface of the ventricle. In discussing the vascular arrangement of the myocardium, Grayson *et al.* (1974) note that the numerous arterioarterial anastomoses seen on the epicardial surface of the heart did not seem to be protective against acute myocardial infarction. The network of vessels seen at the precapillary level appeared to be very similar to that seen in skeletal muscle. The primary subepicardial network of vessels was found to be the main origin of the microcirculation and also gave rise to the main capillary beds of the outer portion of the myocardium. Its perforating branches supplied the endocardium and the septum as well as the bulk of the ventricular muscle.

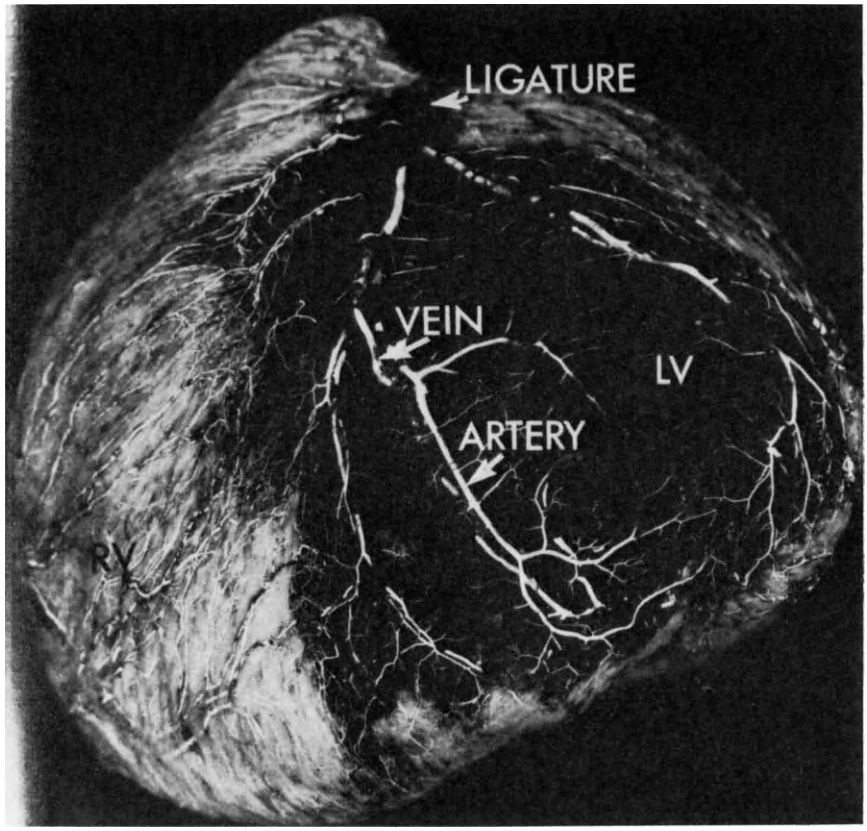

Fig. 10. A dog heart, injected with Microfil, after ligation of the anterior descending coronary artery. Note the black area where Microfil did not enter the capillaries [Grayson *et al.* (1974)].

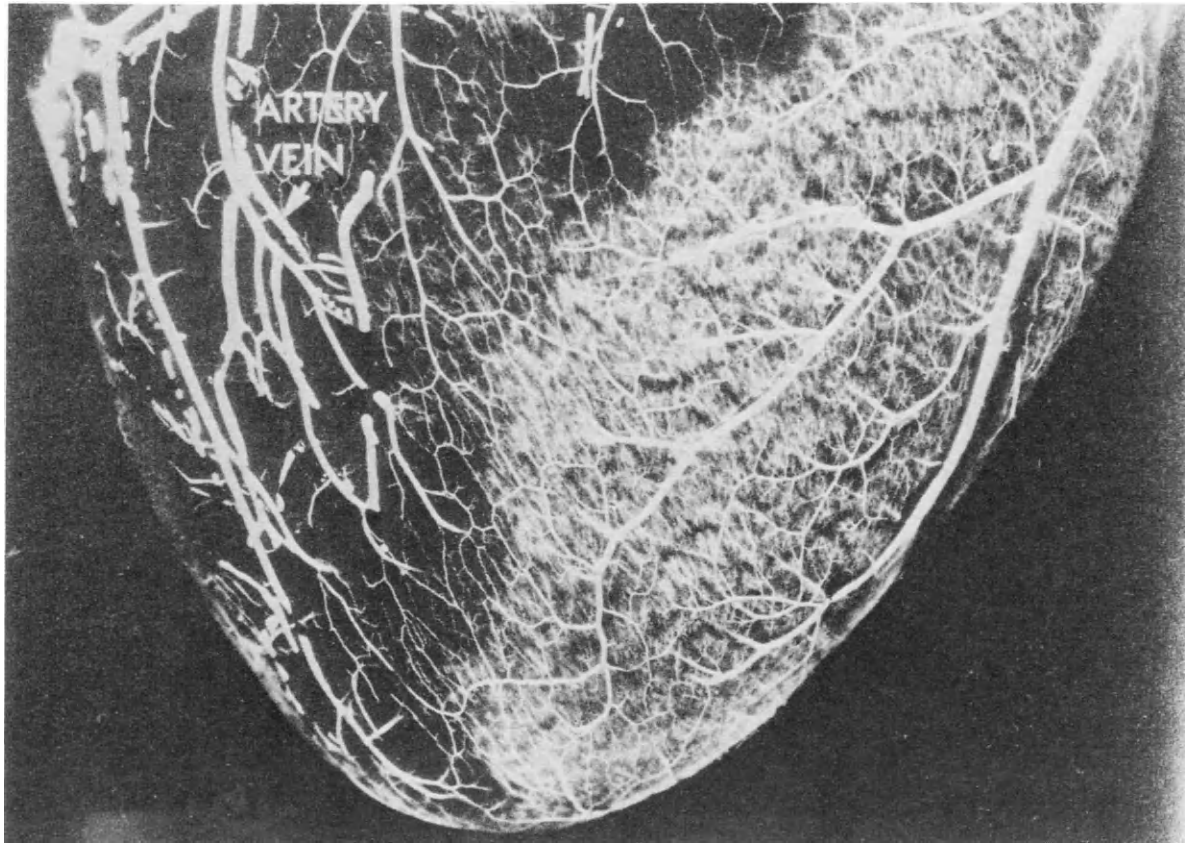

Fig. 11. An infarct in the left ventricle of a dog heart shown by Microfil injection [Grayson *et al.* (1974)].

In discussing the ischemic mechanism, Grayson *et al.* (1974) note that the Microfil, and presumably blood, can be carried by vessels in the infarct down to those vessels that are 20 μm in diameter and these are the ones that account for most of the resistance to flow. It seems remarkable that, although the blood (or Microfil) has passed the major area of resistance, the blood does not enter the capillaries. Failure of blood to perfuse the tissue has been explained simply as the blood being under an inadequate head of pressure. Local reflexes or alterations in vascular responses to circulating catecholamines have also been used in attempts to explain the observation but such responses are not confirmed. The explanation will most likely be found in the alterations in hemodynamics that occur in low flow states.

The requirements for the use of tracer microspheres to measure regional myocardial flow were tested by Flameng *et al.* (1977). They compared the results in normal hearts with low coronary flow produced by ligation of the circumflex branch of the left coronary artery using six differently labeled microspheres in quantities ranging from 550,000 microspheres/100 g of heart weight to 11,000 microspheres/100 g of heart weight. Their results showed that in dog hearts the local myocardial flow could be measured in tissue samples of about 400 mg at the extreme inhomogenous flow resulting from coronary occlusion if at least 110,000 spheres/100 g of tissue enter the coronary vascular system. At least 345 spheres per sample were need for a mean deviation of 10%. Caution must be used in accepting the accuracy of the method in low flow states in any size animal, and the accuracy may be questioned in normal flow states in small animals such as rats.

In 1977, Turek *et al.* described right cardiac hypertrophy after a left-sided myocardial infarction in rats. The right ventricular hypertrophy resulted in an increase in weight of the ventricle that was twice as great as controls. Histological sections showed muscle fiber diameters and diffusion distance to be much larger in the rats with the infarctions. No change in tissue oxygen was seen in animals 4.5 weeks after ligation of the left coronary artery. Capillary diameter (external) was increased and capillary density decreased as did the number of muscle fibers per square millimeter in the hypertrophied right ventricle. The larger diffusion distance most likely would result in an impairment of tissue oxygen transport in the hypertrophied muscle.

These investigations have shown that there are different auxiliary pathways for various areas of cardiac muscles, that capillaries in ischemic areas resist perfusion to reestablish flow, that marked changes in flow after coronary occlusion require careful consideration of the method of measuring regional distribution of blood flow, and that capillary

diameters and muscle fiber size change in a hypertrophied right ventricle produced as a result of a left-sided myocardial infarction.

The most general statement that can be made is that the capillary beds appear to suffer almost irreparable damage as a result of the deprivation of blood flow brought about by interference with their source of supply of blood. Most indicative of the degree of damage is the difficulty in reinstituting flow through the capillaries. Based on information garnered from other studies of the microcirculation, speculation as to the causes might include any number of changes. When perfusion pressure of the beds is reduced, accumulation of the cellular components can be fostered. Platelet aggregates can form more easily and effectively block capillary circulation. Red cell aggregates or rouleaux are also known to form in low flow states to produce stasis. Leukocytes have long been known to occlude capillaries because of their large size and their rigidity. They also become more adhesive as velocity of flow decreases. In addition to a further reduction in flow caused by constituents of the blood, changes such as increased permeability of capillary vessels must be considered. If this is a consequence of ischemia, and outward filtration is enhanced, then local edema must be another abnormal condition to interfere with circulation through the terminal vascular beds. Undoubtedly, the ability of the arteriolar vessels to respond to the stimuli that normally evoke autoregulation must be diminished. The alternative, unless flow can be reestablished by inhibiting the occurrence of these damaging events, is to depend on the rapid establishment of collateral circulation, either immediately by opening up previously nonperfused pathways or subsequently by awaiting neovascularization.

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2 Animal Studies in Artificially Induced Myocardial Infarction

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I. INTRODUCTION

Myocardial infarction (MI) and coronary heart disease are still the leading causes of death in this country. The two serious consequences

Myocardial Infarction and Cardiac Death

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that result from occlusion of the coronary arteries and the resultant MI are (1) the development of life-threatening arrhythmias and (2) the failure of the heart as a pump.

One of the immediate consequences of coronary occlusion is the severe ventricular rhythm disorders which account for most of the deaths in the hours immediately following an acute myocardial infarction. About 650,000 people die each year from coronary heart disease culminating in myocardial infarction. The introduction of the coronary care unit (CCU) has resulted in a dramatic reduction of nearly 30% in the mortality of acute MI patients in the hospital (Lown and Wolf, 1971). These investigators also pointed out, however, that the universal availability of the CCU would probably only reduce the in-hospital mortality by another 10%. The reason why greater numbers with acute MI cannot be helped by the CCU is that 60–70% of MI related deaths occur in the first hour after the onset of symptoms, before they even reach the hospital. It is thought that most of these patients succumb as a result of ventricular arrhythmias. With the advent of the coronary care unit, much investigation has been performed to elucidate the mechanisms involved in the rhythm disturbances and to develop agents that reverse the rhythm disturbance or prevent its recurrence.

The second serious consequence of acute MI, pump failure, has also received a great deal of attention in recent years. With the development of techniques to measure infarct size in experimental animal models and in patients, emphasis has been placed on protecting the ischemic myocardium from further damage by limiting the extent of the ischemic injury. It is now well known that the seriousness of the hemodynamic sequelae of myocardial infarction is related directly to the amount of myocardium that is lost to infarction. Numerous pharmacologic agents and therapeutic modalities have been found that decrease the ultimate size of an infarct, thereby leaving the patient with a greater quantity of viable myocardium.

The purpose of this chapter is not to provide an exhaustive review of all of the experimental data relating to arrhythmia and infarction, but to provide the reader with an appreciation of the animal models used to examine the complex interrelationships between myocardial ischemia, infarction, and arrhythmia. We will, where appropriate, point out advantages and disadvantages of animal models for the particular parameter being evaluated. It is important to point out that there are very few studies available offering a direct comparison of animal models for infarction and arrhythmia. The subject of a need for appropriate animal models to study the sequelae of myocardial infarction has been addressed previously in symposia organized by the National Institutes of

Health (NIH) as well as in a monograph by Schaper (1980). Ultimately, it will become apparent to the reader that there is no single animal model ideally suited to examine all of the parameters that need to be studied. In fact, lack of agreement over which animal models to use led to a request by the National Heart, Lung, and Blood Institute of NIH for proposals aimed at the "characterization and evaluation of animal models for use in the assessment of therapy to protect ischemic myocardium." This call for proposals, moreover, restricted the studies to only two species: the rat and the dog.

It is with sincere apology that the authors note that it is impossible in a work such as this to cite every study published on this important subject and that representative studies only are used to highlight critical areas. After completing this chapter, the authors hope the reader will be better prepared to examine more critically the voluminous literature pertaining to the use of animal models in myocardial infarction.

II. METHODS OF PRODUCING CORONARY ARTERY OCCLUSION

There are a variety of techniques by which blood flow throught the coronary vasculature can be restricted resulting in cardiac ischemia. The relationship of the various techniques to the clinical situation is a matter of continuing controversy. The most commonly employed method involves the placement of a ligature around the coronary artery [usually the left anterior descending (LAD) or circumflex] in anesthetized animals which, when tied, results in total occlusion of the coronary artery. There are two disadvantages of this technique: (1) the animal's chest must be opened surgically, resulting in hemodynamic changes and necessitating ventilation of the animal, and (2) the animal is anesthetized for the surgical manipulation. In many studies one finds that the ventilation parameters are not mentioned. Even when these parameters are given, many investigators fail to control arterial blood gasses and pH.

Two techniques that can be performed with a closed chest to overcome the first limitation are the use of a balloon catheter, and mercury, agar, or glass bead injection. With the former, a catheter containing an inflatable balloon at the tip is guided under fluoroscopy into the desired coronary artery. The artery is occluded by inflating the balloon, blocking all blood flow distal to the inflated balloon. The second tech-

nique also involves the use of a catheter placed in the coronary artery under fluoroscopy. When a bolus of mercury, agar, or glass beads is injected through the catheter into the vasculature there is a diffuse interruption of the blood supply to the affected myocardium. For the most part, techniques utilizing the placement of catheters in the coronary artery are most easily performed in large animals (i.e., dog or pig).

The disadvantage that remains even with the closed chest operation is the use of anesthesia, which plays an important part in the sequelae following coronary artery occlusion. It is now appreciated that the cardiovascular reflexes play a critical role in the response of the animal to acute occlusive episodes and that anesthetic agents vary in their capacity to alter autonomic reflexes; that is, they may enhance or depress these reflexes. Moreover, evidence from Vatner and colleagues (1974) clearly shows that anesthesia alters the response of the animal to a variety of pharmacologic agents commonly employed in the ischemic setting. Franciosa *et al.* (1977) observed in the dog that anesthesia reversed some of the hemodynamic responses to acute coronary artery occlusion. Clearly, a conscious animal would yield results more closely applicable to the clinical arena. This concern has been addressed by performing coronary occlusion in chronically instrumented awake animals. Both coronary embolization with mercury and coronary occlusion using aneroid constrictors have been performed in conscious animals (Franciosa *et al.*, 1977; Jugdutt *et al.*, 1979). The surgical procedure during which either a catheter or aneroid constrictor is put in place in or around the coronary artery is conducted under general anesthesia while the occlusion is produced many days later in the conscious animal. Thus, interventions to alter infarction and arrhythmia can be tested with intact cardiovascular reflexes in the absence of anesthetic agents.

In the techniques described above, the occlusion produced is usually permanent; yet, patients dying from acute MI are frequently found to have patent coronary arteries. This postmortum observation suggests that the occlusion of the coronary artery was transient, possibly resulting from spasm of the vascular smooth muscle within the vessel wall. Therefore, techniques have been developed in animals to simulate this event. Complete occlusion of the coronary vessel is produced with the ligature or balloon catheter technique. The occlusion is maintained for a predetermined period of time ranging from minutes to hours to allow for the development of ischemia and/or infarction. At the appropriate time, the occlusion is removed, restoring flow to the affected area. This technique, usually referred to as reperfusion, results in an immediate, marked increase in the incidence of ventricular fibrillation. Both the

dog and the cat show a similar response to reperfusion (i.e., increased arrhythmia and mortality). With the bolus mercury injection or the aneroid constrictor technique, reperfusion of the coronary vasculature is not possible.

The techniques described above are generally believed to be non-thrombotic; that is, they do not involve the deposition of platelets to produce occlusion in the coronary vasculature. In order to generate a model involving platelet aggregation, investigators have employed a partial occlusion of the coronary artery, which initiates the formation of a thrombotic plug. In this situation, incomplete occlusion is produced by partially constricting the coronary artery using a ligature resulting in the rapid accumulation of a platelet plug, which eventually produces complete occlusion (Folts *et al.*, 1976). Some investigators feel that this model more closely resembles the actual pathological process encountered in the clinical situation with occlusive coronary artery disease. In addition to a partial occlusion, another technique used to produce a thrombotic occlusion involves the administration of arachidonic acid or thromboxane into the coronary vasculature (Shimamoto *et al.*, 1977). Arachidonic acid is converted by the platelets into thromboxane, which greatly accelerates platelet aggregation, resulting in the rapid formation of a platelet plug occluding the vessel. Finally, in rodents, cauterization of the coronary artery has been employed. This technique produces a diffuse area of injury resulting in complete occlusion (Ikram *et al.*, 1975).

During the first hour following abrupt, complete coronary artery occlusion in animals, ischemia develops resulting in a high frequency of arrhythmias, often culimnating in mortality. This period has frequently been compared to the prehospital phase of myocardial infarction in humans where 60 to 70% of the deaths resulting from MI are believed to occur. Thus, the abrupt occlusion models can be employed to evaluate the biochemical, hemodynamic, and electrical consequences of ischemia. The acute occlusion techniques have been used most often in dogs; however, there is increasing interest in other species. Several studies have been reported using the cat (Gillis, 1971; Lathers *et al.*, 1978; Ritchie *et al.*, 1979; Spath *et al.*, 1979; Kelliher *et al.*, 1979), and there is also continued interest in the use of the rat as a model for acute occlusion (Fishbein *et al.*, 1980). Far fewer studies have been conducted in the pig (Most *et al.*, 1974, 1976; Holland and Brooks, 1975, 1977), rabbit (Shimamoto *et al.*, 1977), and baboon (Bruyneel and Opie, 1973; Lubbe *et al.*, 1974; Fore *et al.*, 1978).

The techniques described above all result in abrupt interruption in blood flow, subsequently producing arrhythmias and death in a high

percentage of the animals employed. There are many instances, however, when an investigator wishes to study events occurring hours or days after occlusion, (e.g., arrhythmias, infarction, sudden death). This can be accomplished by using one of the acute occlusion techniques described above and then studying the survivors of this procedure. This is not an uncommon practice. The disadvantage of this approach is, of course, the high mortality occurring in these animals during the first hour following occlusion. In order to avoid this high incidence of mortality and to study the events occurring at the later time period after occlusion, several techniques have been employed. The most commonly employed procedure was originally described by Harris (1950). In this situation, the coronary artery is occluded in two stages over a period of approximately 20 min. The first stage consists of a ligature that occludes the artery by approximately 50–60%. The second stage occurs 15–20 min later, at which time the artery is totally occluded. This procedure significantly reduces the mortality associated with abrupt occlusion; however, arrhythmia does develop several hours later (Fig. 1). As with the earlier techniques described, an open chest anesthetized animal is required initially; however, following recovery from surgery the animal can be followed for days, weeks, or even months. There are many variations on the original description of this technique published by Harris, but the important point common to all

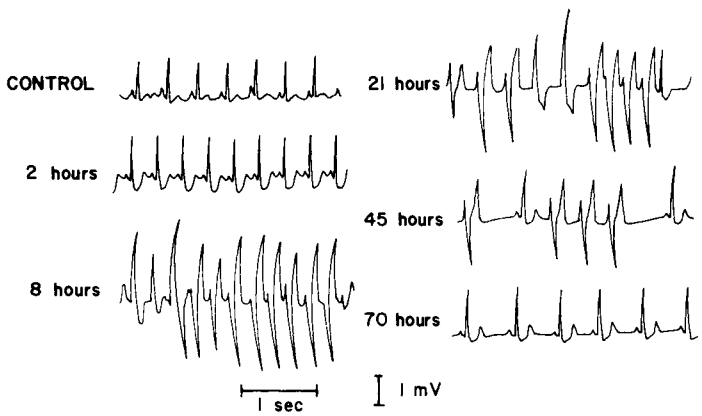

Fig. 1. Development of the delayed phase (late) of arrhythmia in the dog. Lead II ECG tracings (retouched) were obtained from a dog immediately prior to and an various times after two-stage ligation of the left anterior descending coronary artery (LAD) at the level of the tip of the left atrial appendage. Ectopic frequency was low until 4 h after coronary ligation. Peak ectopic activity existed during 8–24 h, then the number of ectopic beats gradually decreased. At 70 h, most beats were normal, sinus in origin. Similar results were obtained in all five dogs in which the LAD was ligated.

procedures is that the blood flow in the coronary artery is reduced gradually over a period of 15 to 20 min rather than abruptly, as described in the preceding section. Studies using this procedure have been reported most often in the dog, although the cat has also been used (Ritchie *et al.*, 1979). It should be noted that there are important differences between the dog and cat with respect to the development and nature of the late arrhythmia (see Section III for a more complete discussion). This slow occlusion procedure has not been used extensively in the rat or pig. Slow occlusion can also be produced in a conscious, unanesthetized animal using the aneroid constrictor.

III. VENTRICULAR ARRHYTHMIA ASSOCIATED WITH MYOCARDIAL INFARCTION

One of the immediate consequences of ischemic injury to the myocardium is the development of severe ventricular rhythm disturbances that account for most of the deaths in the hours immediately following an acute MI. Of the 650,000 Americans who die each year from coronary heart disease culminating in MI, 60–70% of them die in the first hour after the onset of symptoms before they even reach the hospital. It is thought that most of these patients succumb as a result of ventricular arrhythmias. In this regard, it has been shown that following an MI almost all patients exhibit ventricular arrhythmia, with 70 to 80% of these individuals requiring pharmacological therapy (Mogensen, 1970). Numerous studies have demonstrated that ventricular ectopic beats predispose the heart to ventricular tachycardia and ventricular fibrillation (VF) or sudden death (Chiang *et al.*, 1969; Lown and Wolf, 1971; Kotler *et al.*, 1973; Coronary Drug Project Research Group, 1973; Moss *et al.*, 1974; Vismara *et al.*, 1975). Thus, two approaches to decreasing mortality further are (1) reaching the acute MI patient sooner, and this is now being done with the advent of the mobile coronary care unit (Pantridge and Adgey, 1969), and (2) treating effectively the ventricular rhythm disturbances that occur in the first hour following myocardial infarction, allowing time for the patient to reach the coronary care unit. The latter approach requires the development and use of experimental animal models to study the underlying mechanisms responsible for the genesis of ventricular arrhythmia, and to develop antiarrhythmic agents that would be useful in the clinical setting.

Numerous mechanisms are probably involved in the development of the severe electrical disturbances in cardiac rhythm that follow acute MI, including: neural, biochemical, anatomical, and temporal factors. In the following section we will describe the importance that each of these factors may have in relation to the different animal models employed for studying occlusion-induced arrhythmias.

A. Temporal Factors Involved in Occlusion-Induced Arrhythmia

The time that it takes to totally occlude a coronary artery and the time after coronary artery occlusion when cardiac rhythm is monitored appear to be important factors in the development of arrhythmia. In 1950, Harris defined three stages of arrhythmia following abrupt occlusion of the left anterior descending coronary artery in the anesthetized dog. In the first phase, ectopic activity began within 2 to 5 min following abrupt occlusion, increased in frequency, and either precipitated into ventricular fibrillation or passed through a maximum rate and reverted to a sinus rhythm within 15 to 30 min. This is commonly referred to as the early phase of arrhythmia and is thought to correspond in humans to the period immediately after the onset of symptoms before patients reach the hospital or its coronary care unit. The second phase is a period of little ectopic activity, which begins with the decline of ectopic frequency that occurs in the first phase and lasts until the start of ectopic activity in the third phase. The third phase begins approximately 4.5–8 h after coronary artery occlusion and is characterized by a sudden, rapid onset of ectopic ventricular complexes, reaching a peak frequency at 15 to 30 h, then declining but persisting for 2–3 days. This is referred to as the late phase of arrhythmia and is thought to correspond in humans to the period after occlusion when the patient would be in the coronary care unit.

The period of early arrhythmia following coronary artery occlusion has been studied in the dog, cat, rat, pig, rabbit, and baboon. The delayed or late arrhythmia (phase 3) has been studied for the most part in dogs. Studies of late arrhythmia in the rat and pig are scarce. It is important to point out that the cat is probably not a suitable animal in which to study the mechanism underlying late arrhythmia. This is based on the finding that, unlike the dog, cats do not develop the delayed phase of arrhythmia following two-stage LAD occlusion. A comparison of the effects of this procedure in dogs and cats has been

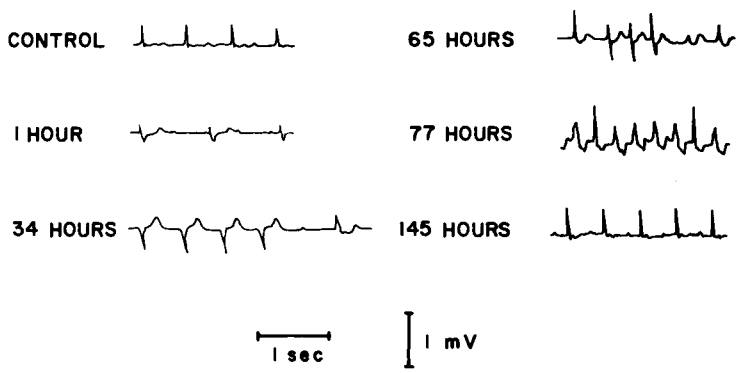

Fig. 2. Development of the delayed phase (late) of arrhythmia in the cat. Complete heart block was produced surgically 1 week before the experiment. The control panel is of a Lead II ECG obtained after heart block was produced, but before coronary artery occlusion. The other panels represent the ECG obtained at the hours indicated after two-stage occlusion of the LAD. Note the appearance of ectopic activity at 34, 65, and 77 h as indicated by the change in ventricular rate and configuration of the waveform. The recording speed was 25 mm/sec and all recordings were obtained while the animal was conscious. Late arrhythmia does not occur in the cat unless complete A–V dissociation is present (Reynolds *et al.*, 1979).

published (Reynolds *et al.*, 1979). Briefly, these studies show that [during several days following two-stage LAD occlusion] ventricular arrhythmia is not present in cats at a time when the dog shows nearly a 100% incidence of ectopic complexes. Late arrhythmia can be produced in the cat with LAD occlusion, however, only when complete A–V block is produced prior to occlusion of the coronary artery. An example of late arrhythmia in the dog and cat is depicted in Figs. 1 and 2. A period of arrhythmia occurring several weeks to months after LAD occlusion has been described in the cat by Myerberg *et al.* (1977). Taken together the studies indicate that the time after occlusion when the arrhythmia is evaluated is an important factor as well as the time that it takes to completely occlude the artery.

B. Neural Factors Involved in Occlusion-Induced Arrhythmia

There is ample evidence in the literature based on data obtained for the most part in dogs indicating the mechanisms involved in the development of early and late arrhythmia following coronary artery occlu-

sion are different. In order to alleviate the high incidence of ventricular fibrillation (30–35%) that occurs during the first phase following abrupt occlusion, Harris developed a model of two-stage slow coronary occlusion, which we have discussed previously in Section II. This model reduced the first phase (early arrhythmia) with its high mortality and enabled Harris to better characterize the late phase of arrhythmia, which occurred 4.5–8 h after coronary occlusion and lasted for 2 to 3 days. Studies with the early and late arrhythmia model led to the appreciation of mechanistic differences between early and late arrhythmia, which will become apparent in the following paragraphs.

There are numerous reports to demonstrate that the autonomic nervous system is involved in the development and severity of ventricular arrhythmia following coronary artery occlusion in humans. In one clinical report, Webb *et al.* (1972) found that 92% of the patients seen within 30 min of the onset of symptoms of acute MI displayed prominent alterations in autonomic nervous activity, both sympathetic and parasympathetic. In this regard, as an expression of increased sympathetic activity, it was found that both plasma and urinary catecholamine levels are elevated following acute MI (Forssman *et al.*, 1952; Nazum and Bischoff, 1953; Gazes *et al.*, 1959; Videbaek *et al.*, 1972). Moreover, it has also been shown that a higher incidence of arrhythmia is associated with these increased levels of catecholamines (Januszewicz *et al.*, 1968; Jewitt *et al.*, 1969; Lewis *et al.*, 1972).

Because of the aforementioned prevalence of autonomic nervous activity in the setting of acute MI and its importance as a determinant of arrhythmia, many studies have been conducted in animal models to ellucidate the contribution of the autonomic nervous system to the development of cardiac rhythm disturbances. Many studies indicate that sympathetic innervation to the heart has an important influence on arrhythmias and death occurring within the first hour following acute coronary artery occlusion. The earliest evidence was based on the observations that removal of adrenergic nervous activity to the heart afforded protection against the lethal rhythm disorders resulting from acute coronary artery occlusion. Harris *et al.* (1951) reported that bilateral stellate ganglionectomy greatly reduced the early ventricular arrhythmia and mortality occurring in the first 15 min following coronary artery occlusion in dogs, but failed to alter the late arrhythmias that began 4–8 h after occlusion. They suggested that only during the first phase is sympathetic nervous activity of actual importance to the development of arrhythmia and death following coronary artery occlusion. Some time later, Ebert *et al.* (1968) and Schaal *et al.* (1969) demonstrated in dogs that depletion of cardiac catecholamine stores

produced by chronic cardiac denervation afforded protection against the development of postocclusive ventricular rhythm disorders. Gillis (1971), employing the anesthetized cat, also found that sectioning of the spinal cord at the level of the first cervical vertebrae along with bilateral vagotomy conferred protection against arrhythmias resulting from occlusion of a major coronary artery. He reported that animals with no neural input to the heart survived more than twice as long after occlusion and showed a significant reduction in the incidence of ventricular fibrillation. In addition to surgical procedures, sympathectomy produced by pharmacologic means has also been observed to reduce fatal arrhythmias occurring within the first hour following occlusion in dogs (Maling *et al.*, 1959; Grayson and Lapin, 1966; Bacaner and Schrienemacher, 1968; Khan *et al.*, 1972) and cats (Gamble and Cohn, 1972; Corr and Gillis, 1975).

The importance of neural activity in the arrhythmias following occlusion has been evaluated in studies involving direct measurement of sympathetic nerve activity. Constantin (1963) found in dogs that adrenergic nervous activity was reduced following coronary artery occlusion. More direct evidence that sympathetic nerve activity is involved in the development of arrhythmia after coronary occlusion comes from studies by Malliani *et al.* (1969). These authors reported that brief periods (1–2 min) of coronary artery occlusion in cats resulted in altered discharge patterns recorded from preganglionic sympathetic nerve fibers. They found that sympathetic nerve activity not only increased after coronary occlusion, but also, in some fibers it decreased, or did not change at all. In each case, however, when a change in nerve activity occurred, it did so prior to the development of arrhythmia. When the occlusion of the coronary artery was relaxed, spontaneous activity in the sympathetic nerve fibers returned to preocclusion levels and cardiac rhythm returned to normal. The majority of the studies in which nerve activity was recorded have been conducted using the cat as a model for coronary occlusion. In 1971, Gillis, recording from preganglionic sympathetic nerve fibers, in chloralose-anesthetized cats, noted both increases and decreases in the rate of discharge following permanent occlusion of the left coronary artery. It has been reported recently (Lathers *et al.*, 1978), again using the chloralose-anesthetized cat but recording from a multifiber preparation, that alterations in sympathetic nerve activity occur in the first 30 min after LAD occlusion and that these changes are associated with the development of ventricular arrhythmias and ventricular fibrillation. An example from data obtained in these studies is depicted in Fig. 3. Earlier investigations have used both the anesthetized dog and cat as models for coronary

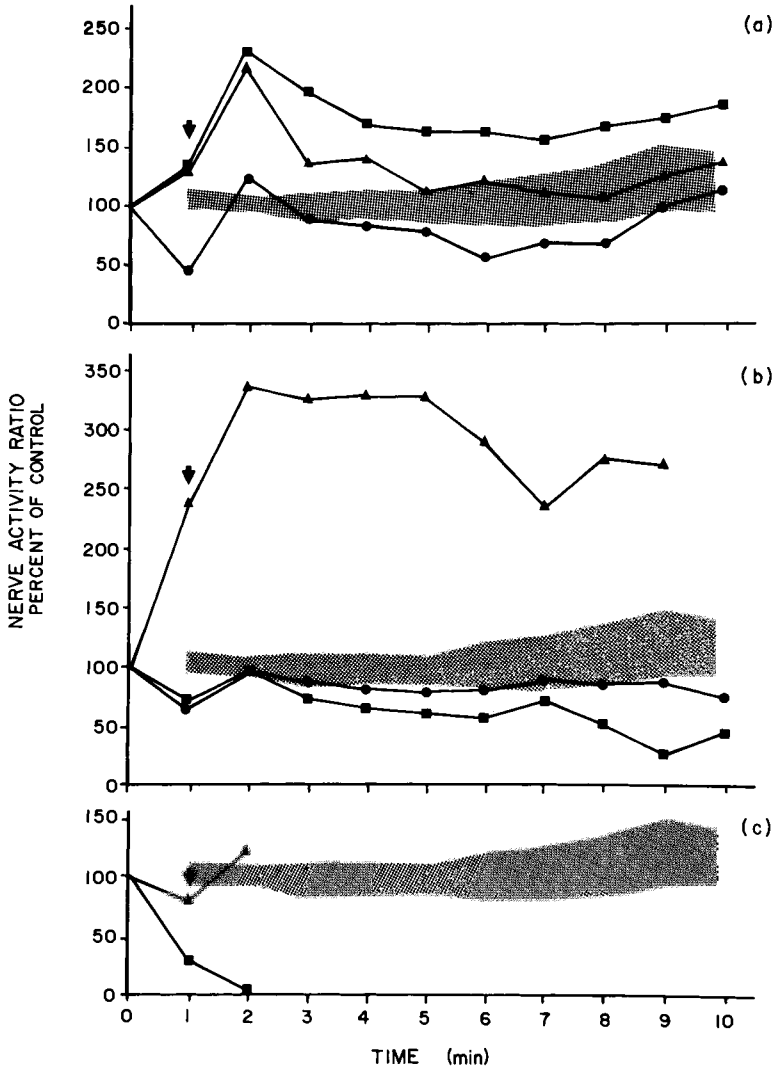

Fig. 3. Effect of coronary occlusion on sympathetic neural discharge. Recordings of spontaneous nerve discharge were obtained simultaneously from two or three postganglionic cardiac sympathetic branches in three cats anesthetized with α-chloralose. The nerve activity ratio depicted on the ordinate was calculated by dividing the mean of the range of spontaneous nerve discharge during a 1-min interval by the existing mean arterial blood pressure. The nerve activity is expressed as a percent of control and is graphed as a function of time. The shaded area represents the 95% confidence limits of the mean nerve activity ratio in a control group of four animals. The arrows in the graph indicate the occurrence of initial arrhythmia. [Reprinted by permission of the American Heart Association, Inc. *Circulation* **57**(6), 1058–1065, (1978).]

artery occlusion. More recently, Lown and colleagues (Kolman *et al.*, 1975) using conscious, unanesthetized dogs have also shown the importance of autonomic neural imbalance in the development of ventricular arrhythmias following coronary artery occlusion.

Another reflection of enhanced sympathetic activity is catecholamine release from the adrenal medulla. This has also been implicated in the genesis of arrhythmia following coronary occlusion. Large increases in plasma epinephrine released from the adrenal gland have been reported in both dogs (Staszewska-Barczak and Ceremuzynski, 1968) and cats (Kelliher *et al.*, 1975) immediately after coronary occlusion. In dogs, Ceremuzynski *et al.* (1969) found that 85% of the animals secreted epinephrine as the principle catecholamine. They also found that in dogs that failed to secrete catecholamines and showed no ectopic activity after coronary artery occlusion, that intravenous infusion of epinephrine provoked ventricular arrhythmias. However, because epinephrine in doses similar to those excreted after occlusion did not produce arrhythmia in dogs without occlusion, the development of ventricular arrhythmia appears to be related to the combination of coronary artery occlusion and increased plasma catecholamine levels. Studies in cats have shown that adrenal vein ligation prior to LAD occlusion did not alter the pattern of ventricular arrhythmia but it did diminish the incidence of VF after coronary occlusion (Kelliher *et al.*, 1975). This suggests that epinephrine released from the adrenal gland may be more important in the development of VF as opposed to nonlethal ventricular arrhythmias. Taken together, this evidence suggests that sympathetic nervous activity and secretions from the adrenal medulla have an important influence on the production of arrhythmia and death in the immediate period following acute coronary artery occlusion. The time after occlusion at which the sympathetic influences are evaluated is important since the nature of this factor seems to decline with time after occlusion.

In addition to the sympathetic nervous system, increases in parasympathetic nervous system activity have also been observed in both humans and animals following coronary occlusion. Recent clinical data indicates that the incidence of bradycardia is very high after MI and this may contribute to the development of VF (Pantridge *et al.*, 1975). In experimental studies, increased vagal activity may be protective or deleterious, depending upon the animal studied and the time interval after coronary occlusion. In this regard, vagal stimulation in the dog is associated with reduced arrhythmia (Scherlag *et al.*, 1970) and increased VF threshold (Kent *et al.*, 1973). In the cat, increasing vagal tone does not have a similar effect (Lawrence and Kelliher, 1975).

The cat exhibits increased vagal tone after coronary occlusion, unlike the dog (Peterson *et al.*, 1973), and increasing this influence does not provide additional protection. Corr and Gillis (1974) demonstrated in the cat that surgical vagotomy or administration of atropine both decreased the time to ventricular arrhythmia and increased the incidence of VF following coronary occlusion. Again, parasympathetic influences seem to be important only during the first hour after occlusion and the importance of this factor is diminished with time after occlusion.

Studies evaluating the importance of the autonomic nervous system in the production of ventricular rhythm disturbances following acute coronary artery occlusion have been performed for the most part in anesthetized dogs and cats. Data from studies in other species such as the pig or rat are not nearly as extensive. Two papers in the literature have probed the role of the sympathetics in the production of postcoronary occlusion arrhythmias in the rat. Kenedi and Losonci (1973a) found that arrhythmias following ligation of the left coronary artery were increased by pretreatment with isoproterenol, a sympathomimetic agent. Pretreatment of rats with several β-adrenergic receptor blocking agents reduced the frequency of occlusion-induced arrhythmias (Kenedi and Losonci, 1973b). Specifically, propranolol, pindolol, and practolol were tested. Propranolol was found to possess the strongest antiarrhythmic effect; thus, the sympathetic nervous system appears to be partially responsible for the generation of coronary occlusion-induced arrhythmias. Despite the antiarrhythmic effects of these agents, survival was enhanced only in the pindolol pretreated rats; the negative inotropic effects of both propranolol and practolol appear to be responsible for their increased lethality.

In our laboratory we have studied the influence of the sympathetic nervous system on the incidence of VF in rats following acute LAD occlusion (Table I). This was accomplished by producing chemical sympathectomy with 6-hydroxydopamine (6-OHDA), an agent that depletes catecholamines by destroying sympathetic nerve terminals (Thoenen and Trazer, 1968). Pretreatment of rats with 6-hydroxydopamine was used to demonstrate the sympathetic nervous system's role in the pathogenesis of arrhythmias while avoiding any chronotropic or inotropic side effects of β-adrenergic blocking agents. Although the results of the experiments are for the most part statistically inconclusive, trends appear to exist between 6-OHDA pretreatment and the following: (1) a decreased frequency of observed PVC/min, (2) a decreased frequency of rats experiencing ventricular tachycardia ($p < 0.05$), (3) a similar decrease in the frequency of rats experiencing ventricular flutter or fibrillation, and (4) an increased incidence of

TABLE I
Effect of Removing Autonomic Nervous Influence on Arrhythmia and Death following Coronary Artery Occlusion in the Rat

Pretreatment	Saline	6-Hydroxydopamine	Atropine
N	8	8	7
PVC/min ($\bar{x}$ ± S.E.)[a]	44.3 ± 8.1	16.1 ± 2.8	63.3 ± 8.5
Ventricular tachycardia (%)	100	38[b]	100
VF (%)	50	25	86
Heart block (%)	25	63	0

[a] Significant differences exist between groups, $p < 0.005$, determined by analysis of variance with post hoc Student's *t*-test.

[b] Significantly different from control, $p < 0.05$; Fisher's exact test.

heart block not immediately precipitated by ventricular tachycardia (VT) or VF. The first three trends seem to imply that the intact sympathetic nervous system plays a role in the genesis of PVCs and ventricular flutter or fibrillation in the rat. The trend between 6-OHDA pretreatment and an increased incidence of heart block implies that normal atrioventricular nodal impulse conduction requires sympathetic nervous system input in the rat. Although some of these heart blocks may have resulted from atrioventricular (AV) node ischemia due to septal artery occlusion with left coronary artery occlusion (Halpern, 1957), heart block still appears to be more prevalent in the 6-OHDA treated rats than in the vehicle treated rats. In all the observed 6-OHDA treated rat heart blocks, the atrial rate after heart block was no greater than the initial heart rate and exceeded the ventricular rate after heart block; this probably indicates that normal impulse conduction through the AV node was sufficiently slowed by the decrease in cardiac sympathetic tone to cause a ventricular ectopic pacemaker to emerge. Thus, unlike man, the AV node of the rat appears to function normally with a tonic sympathetic activity (James, 1969).

The influence of the parasympathetic nervous system on the incidence of VF in rats following acute LAD occlusion was also studied in our laboratory. This was accomplished by pretreating the rats with atropine (Table I). Atropine treatment failed to alter (1) the frequency of PVC/min and (2) the frequency of rats experiencing ventricular flutter or fibrillation. The results imply that the parasympathetic nervous system does not play a role in the genesis of PVCs and ventricular flutter or fibrillation in the rat. Thus, the rat heart appears to function normally under a discernable sympathetic tone. Man's heart, however, appears to function normally under a parasympathetic tone (James, 1969). This

basic difference, as well as the differences in coronary impulse conduction (Prakash, 1954) and circulation (Halpern, 1957; Bloor *et al.*, 1967), must be weighed carefully by any investigator before the rat is used as an antiarrhythmic model for man.

C. Anatomical Factors Involved in Occlusion-Induced Arrhythmia

The anatomic site of occlusion has also been found to influence the rhythm disorders following coronary occlusion. George and Greenwood (1967) and Adgey *et al.* (1971) reported that posterior infarction in patients was associated with a high incidence of bradyarrhythmias. On the other hand, anterior infarction has been shown to result in tachyarrhythmia of ventricular origin. Webb *et al.* (1972) reported that adrenergic activity, as indicated by changes in heart rate and blood pressure, was elevated in the majority of patients who develop infarction of the anterior aspect of the left ventricle. Karlsberg *et al.* (1979) have determined, however, that the magnitude of sympathoadrenal activation after MI in patients was related to the size and not the site of infarction. Additional conflicting reports have been published concerning whether the severity of arrhythmia depends more on the site of occlusion or the size of the infarct in patients. For example, Cox *et al.* (1976) reported that the incidence of ventricular arrhythmias in patients during the first 20 h of hospitalization was related to the estimated infarct size. Yet, Federman *et al.* (1978) found the incidence of ventricular arrhythmias in the first year after MI to be related to the site of coronary occlusion; patients with inferior or posterior infarction had significantly more arrhythmias than patients with anterior infarctions. Infarct sizes in this study, however, were not equal between the patient subpopulations; patients with inferior or posterior infarcts had significantly larger infarcts than patients with anterior infarcts. Thus, in man it appears that both anatomical site of occlusion and infarct size influence the rhythm disorders following coronary occlusion, although the importance of each of these factors alone remains to be clarified.

In general, similar relationships exist with studies conducted in animals. In dogs, both the site of occlusion and the size of infarction influence the rhythm disorders following coronary occlusion. Like man, it is difficult in the dog model to weigh the importance of the site of occlusion against the magnitude of infarction, since occlusion of the same coronary artery at the same site has resulted in significant varia-

tion in the size of the resultant infarcts in several studies (Blumgart *et al.*, 1941; Lowe *et al.*, 1978). Since the dog heart contains an abundant preformed epicardial network (Becker *et al.*, 1973; Cox *et al.*, 1975; Smith *et al.*, 1975; Bishop *et al.*, 1976; Rivas *et al.*, 1976; Hearse *et al.*, 1977), differences in infarct size have been attributed to the variable development of coronary anastomoses and collaterals in the dog heart. Despite this major reservation, Harris (1950) reported a greater incidence of rapid ectopic systoles, but less mortality, in dogs as the distance of the ligation was increased from the origin of the LAD. Allen and Laadt (1950), also using the dog model, reported that the precise level of the ligature on the circumflex coronary artery influenced the incidence of VF. The closer the occlusion was to the origin of the circumflex (i.e., the less the number of branches left unoccluded) the higher the incidence of VF at 1 and 24 h. These studies imply that the size of the area involved after occlusion is a prime determinant of mortality in dogs. But, Thomas *et al.* (1970) reported that, with LAD occlusion in the dog, the severity of arrhythmia depended more on the site of occlusion than on the size of the infarct, since infarcts of approximately the same size yielded great variability in the amount of arrhythmia that occurred. Occlusion of anterolateral branches of the LAD tended to produce less arrhythmia than occlusion of the terminal branch of the LAD, even when infarcts of equal size were produced. However, in our own studies, we found that occlusion of the anterolateral branches of the LAD produced arrhythmia that lasted much longer than that resulting from occlusion of the LAD alone (Reynolds *et al.*, 1979).

Data from studies in other species such as the cat, pig, and rat are not nearly as extensive. Both site of occlusion and size of infarction influence the rhythm disorders following coronary occlusion in the anesthetized cat. Occlusion of the circumflex coronary artery results in a high incidence of bradyarrhythmias (Corr and Gillis, 1974). In fact, we have found that circumflex occlusion in the cat produces varying degrees of A–V block presumably due to reduced blood flow to the region of the A–V node (Lawrence and Kelliher, unpublished observations). Occlusion of the LAD at its origin results in tachyarrhythmias of ventricular origin (Kelliher *et al.*, 1975; Lathers *et al.*, 1978), a reproducible frequency of VF (Kelliher *et al.*, 1975, 1979; Dix *et al.*, 1979), and a reproducible infarct size in survivors (Ritchie *et al.*, 1979). The reproducibility of infarct size in the cat may relate to the finding that, unlike the dog, the cat heart possesses few preformed collateral vessels (Habermehl, 1959). Recent work in our laboratory has found that both the frequency of ventricular ectopic systoles and mortality decrease as

the distance of the ligation from the origin of the LAD was increased (Soifer and Kelliher, unpublished observations). Infarct size also decreased as the distance of the ligation from the origin of the LAD was increased. These studies imply that infarct size may be a major influence on rhythm disorders following LAD occlusion in the anesthetized cat. A significant correlation has been found between the size of infarction in cats with complete heart block and the frequency of ectopic beats at 24 h after LAD occlusion (Ritchie *et al.*, 1979).

The relationships of site of occlusion and extent of ischemia in the development of arrhythmia have not been studied as extensively in the pig and rat models. Occlusion of the LAD in pigs results in tachyarrhythmias of ventricular origin (Most *et al.*, 1974, 1976). Present evidence indicates that acute occlusion of the LAD at its midpoint in the pig results in an extremely high incidence of VF (Opie *et al.*, 1978). This may relate to the findings that, unlike the dog heart, the pig heart possesses few preformed epicardial collateral vessels (Lumb and Singletary, 1962; Lumb and Hardy, 1963; Schaper, 1971; Joensson, 1975). The consequences of this are that the pig heart develops a core of intense ischemia, whereas the ischemic area in the dog heart is less intense and less uniform (Becker *et al.*, 1973; Cox *et al.*, 1975; Smith *et al.*, 1975; Bishop *et al.*, 1976; Rivas *et al.*, 1976; Hearse *et al.*, 1977). In the rat, occlusion of the LAD and great cardiac vein at the origin of the LAD results in tachyarrhythmias of ventricular origin. Virtually the entire left ventricular wall becomes infarcted (Fishbein *et al.*, 1980). In our laboratory, most (64%) rats subjected to LAD occlusion at its origin experienced episodes of ventricular flutter and/or ventricular fibrillation; however, all but 25% of these episodes of ventricular flutter and/or fibrillation spontaneously reverted to a less malignant arrhythmia. Such spontaneous VF reversals are rare in humans, dogs, cats, and pigs. Occlusion of the LAD at points more distal from its origin reduce mortality (Selye *et al.*, 1960). Thus, studies evaluating mechanisms of arrhythmia or efficacy of pharmacologic agents should pay careful attention to the animal species employed as well as the effect produced by altering the site of occlusion in the various models.

D. Biochemical Factors Involved in Occlusion-Induced Arrhythmia

Abrupt coronary artery occlusion results in biochemical changes in the heart which have been shown to correlate with the development

and severity of ventricular arrhythmia. Among the changes associated with coronary artery occlusion that may have an effect on the development of arrhythmia or VF are increased free fatty acids (FFA), catecholamines, extracellular K^+, cAMP levels, and more recently increased prostaglandin (PG) synthesis. The reader is advised that, although these factors are discussed individually, it is quite likely that there is a close interrelationship among the factors, the combined effect of which is to exacerbate cardiac rhythm disturbances.

Increased circulating levels of FFA following coronary occlusion have been detected in both humans (Opie, 1975) and animals (Whitmer *et al.*, 1972; Shug *et al.*, 1975). Opinions differ as to whether the increased plasma levels of FFA found in the early stages of MI may directly cause or precipitate arrhythmias, or whether they are merely a coincidental phenomenon reflecting catecholamine secretion. Increased catecholamine activity in the response to acute MI liberates FFA from adipose tissue (Carlson *et al.*, 1965). High circulating levels of FFA have been correlated with serious arrhythmia in patients with acute MI (Oliver *et al.*, 1968; Gupta *et al.*, 1969; Prakash *et al.*, 1972) while other studies have failed to find this association (Rutenbert *et al.*, 1969; Nelson, 1970). The time after occlusion when FFA levels may correlate with the development of arrhythmia is important. Rowe *et al.* (1975) reported that antilipolytic therapy, which reduces FFA levels, can reduce the incidence of ventricular tachycardia in patients with MI if the therapy is started within 5 h of the onset of symptoms. During this time period, there is an increase in activity in the autonomic nervous system, especially in the sympathetic division (Pantridge and Adgey, 1969). The greatest increase in plasma catecholamine levels occurs in the first hour after the onset of MI (Vetter *et al.*, 1974). Urinary catecholamine values have been reported to show a better correlation with FFA and epinephrine than with FFA and norepinephrine after MI (Gupta *et al.*, 1969). Valori (1970) has shown that infusion of epinephrine is followed by a rise in plasma FFA closely related to the plasma epinephrine concentration, but the relationship with norepinephrine and FFA is not as clear. Thus, increased FFA may be an indication of increased sympathetic nervous system adrenal medullary activity after acute MI and increased FFA might be only one of a series of factors facilitating the development of arrhythmias after MI.

Alteration in the potassium (K^+) balance in the heart in relation to occlusion-induced arrhythmia has received a great deal of attention. The finding that K^+ loss from the heart following a coronary artery occlusion can lead to the development of ventricular arrhythmia was first put forth by Harris *et al.* (1954). They discovered that the onset of

late arrhythmia in the dog coincided with increased coronary venous potassium values. Since then, several other studies (Regan *et al.*, 1967, 1969) have supported this finding and have suggested that altered potassium metabolism could account for the vast majority of clinical arrhythmias following MI (Fisch, 1973). Since myocardial depletion of K^+ requires approximately 2–4 h to become evident (Jennings *et al.*, 1957), it is generally believed that increased extracellular K^+, as found in coronary venous blood shortly after ischemia, is involved in the development of early arrhythmias (Johnson, 1976; Holland and Brooks, 1977). This is supported by the findings of Harris *et al.* (1958), who have shown that intravenous and intra-arterial infusion of potassium salts rapidly produces ventricular ectopic beats and fibrillation in the dog.

The role of cyclic nucleotides in the development of occlusion-induced arrhythmias is also an area of continuing interest. Ueda and Okumura (1971) first suggested that the intracellular accumulation of cyclic AMP (cAMP) that is observed following MI may be involved in the development of arrhythmia. They found that agents which inhibit the enzyme responsible for the breakdown of cAMP (phosphodiesterase) were also capable of producing arrhythmia. Following these reports, other investigators have found increased cAMP levels following coronary occlusion in the baboon (Podzuweit *et al.*, 1978), cat (Corr *et al.*, 1978), and pig (Opie *et al.*, 1979). In each of these cases the rise in cAMP levels occurred during the development of arrhythmia. While these reports indicate a good correlation between the development of arrhythmia and cAMP levels, the majority of evidence is largely circumstantial. In this regard, it has been suggested that increased cAMP levels are secondary to a rise in catecholamines (Opie *et al.*, 1979), potassium (Gerlings *et al.*, 1969), and/or FFA levels (Kurien *et al.*, 1971). Thus, increases in cAMP levels following coronary occlusion may result from other metabolic disturbances occurring simultaneously. Furthermore, the increase in cAMP following coronary occlusion noted in the above animal models has not been seen to the same degree in the dog (Russell and Oliver, 1979). During the development of arrhythmia following myocardial ischemia in this model, increased levels of cAMP were not observed; these arrhythmias were related to glucose and FFA metabolism rather than to cAMP. In support of a direct action of cAMP in other models, Lubbe *et al.* (1976) have demonstrated that dibutyryl-cAMP, which gains access to the intracellular milieu, decreases the VF threshold in rats. In addition, these same investigators report that the fall in VF threshold following coronary occlusion in the same model (the rat) coincides with increases in cAMP in the ischemic zone (Lubbe and Opie, 1978). At the molecular level it

has been suggested that the slow response of cardiac action potentials induced by catecholamines may be mediated by cAMP, since it is well established that cAMP is the second messenger during activation of the beta receptor (Reuter, 1974). Thus, catecholamines and cAMP may be involved in the development of the arrhythmogenic slow response following coronary artery occlusion. While the evidence indicates that, in many cases cAMP may indeed be involved in the development of arrhythmia following coronary occlusion, arrhythmia also develops in models (i.e., dogs) where increased levels of cAMP were absent. Thus, additional studies are needed to elucidate the role of this metabolite in the development of arrhythmia in different experimental models before its role in man can be determined.

More recently, the prostaglandins (PG) have also been shown to be released by the ischemic myocardium following coronary occlusion. In addition, numerous reports have indicated that PG also exerts an influence on cardiac rhythmicity. Whether these effects are beneficial or detrimental appears to depend upon the specific PG involved, the model in which it is used, and the dose and route of administration. Ziljilstra et *al.* (1972) were among the first to show the antiarrhythmic activity of PG of the E series in the dog. Following this report, the effects of various PG were demonstrated on arrhythmias produced in rabbits, rats, and cats as well as in humans (Cantor and Kelliher, 1973; Förster et *al.*, 1973; Hutton et *al.*, 1973; Kelliher and Glenn, 1973; Mann et *al.*, 1973; Mest et *al.*, 1974; Mann, 1976).

In contrast, several investigators have reported that prostaglandins have an arrhythmogenic potential. Koss and Nakano (1974) found that $PGF_{2\alpha}$ produces ventricular bigeminy in cats. Results from our laboratory have shown that $PGF_{2\alpha}$, PGE_1, and PGA_1 demonstrate arrhythmogenic potential when they are administered during coronary occlusion in the cat (Lawrence et *al.*, 1978; Kelliher et *al.*, 1979). More recently, we and others have shown that PGI_2 is also arrhythmogenic following coronary artery occlusion in the cat (Dix et *al.*, 1979) as well as in the rat (Au et *al.*, 1980) and rabbit (Karmazyn et *al.*, 1978). These studies indicate that forms of PG have the potential to be arrhythmogenic when they are administered exogenously during the development of ischemia following coronary occlusion. The reports indicating that prostacyclin (PGI_2) is potentially arrhythmogenic are particularly interesting since this PG has been shown to be the primary PG released from the hearts of rats (DeDeckere et *al.*, 1977) and rabbits (Sivakoff et *al.*, 1979) as well as of man (Kaijser et *al.*, 1978) during myocardial ischemia.

We have examined the relationship between PG release and arrhythmia following abrupt coronary artery occlusion by measuring the

amount of PGI_2 released from the ischemic area of the heart after reperfusion of the LAD 10 min after occlusion. These experiments were performed in cats anesthetized with α-chloralose in which blood samples were obtained simultaneously from the ascending aorta and the great cardiac vein, the vessel that drains the ischemic area of the heart in the cat. The results depicted in Fig. 4 show that in vehicle treated control animals there is a marked increase in PG synthesis and release, as indicated by PGI_2, following the development of ischemia. It should be noted that 79% of the animals developed VF and died within 3 min of the start of reperfusion. In order to determine if this endogenous release of PG during myocardial ischemia was involved in the development of ventricular arrhythmia and death, we inhibited PG synthesis before occlusion and thus removed them as a possible source for the

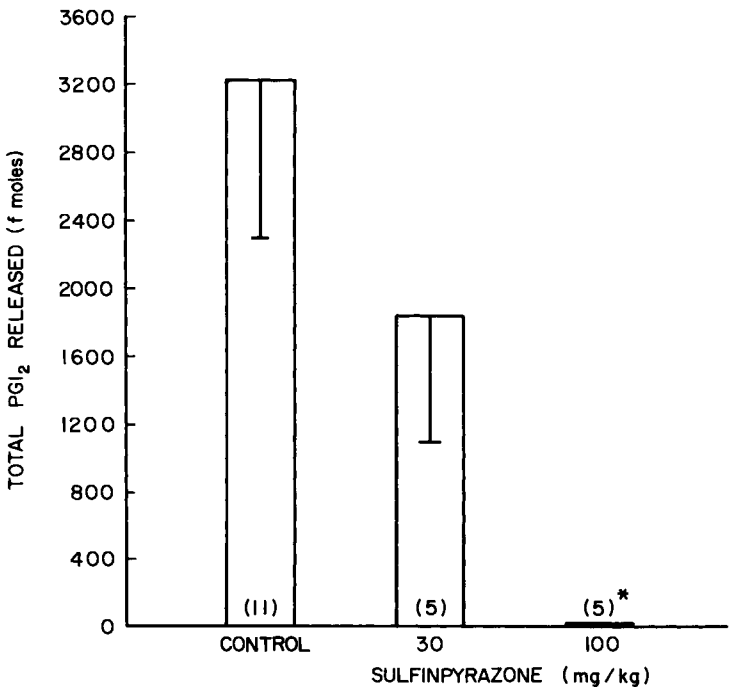

Fig. 4. Levels of prostacyclin (PGI_2) in the great cardiac vein following reperfusion of the coronary artery. PGI_2 levels were measured in plasma obtained from the great cardiac vein in cats anesthetized with α-chloralose. The LAD was occluded for 15 min. Values represent the total amount of PGI_2 in the first 3 ml of plasma from the great cardiac vein following reperfusion; $p < 0.05$ versus sulfinpyrazone (30 mg/kg) and $*p < 0.01$ versus control. Either vehicle or sulfinpyrazone was administered i.v. 1 hr before occlusion. Brackets indicate S.E.M. The number of animals is indicated in the parenthesis and the S.E.M. within the bars.

initiation of arrhythmia and VF. Following the administration of sulfinpyrazone, an agent known to inhibit PG synthesis at the cyclooxygenase step (Flower, 1971), the release of PGI_2 normally observed following coronary occlusion in cats was inhibited. In addition, PG inhibition by sulfinpyrazone was associated with a significant diminution in the amount of ventricular arrhythmia as well as in the incidence of VF in these animals (Kelliher *et al.*, 1980). In addition to inhibiting PG synthesis, this drug also completely eliminated the elevation in plasma norepinephrine levels found in the great cardiac vein in control animals following coronary occlusion. After studying several different mechanisms whereby sulfinpyrazone could exert this antiarrhythmic effect, it was concluded that the most probable mechanism is inhibition of PG synthesis, thereby preventing an arrhythmogenic influence by these substances on the ischemic myocardium (Kelliher *et al.*, 1982). Furthermore, the data indicated that the increased PG levels observed in the cardiac vein were responsible for the elevated norepinephrine levels also observed following coronary occlusion. Thus, the release of norepinephrine following coronary occlusion, and its resultant arrhythmogenic influence, may be dependent upon PG synthesis; that is, the arrhythmogenic influence of PG synthesis is mediated through the sympathetic nervous system. Support for this finding is provided by studies which indicate that the arrhythmogenic (Kelliher *et al.*, 1978) as well as hemodynamic (Ducharme *et al.*, 1968) effects of exogenous PG administration are dependent upon an intact sympathetic nervous system.

Taken together the literature indicates that there are numerous biochemical changes occurring in the heart during the development of ischemia. It is likely that no single change is responsible for the arrhythmia that develops, but a combination or cascade of changes leads ultimately to severe ventricular arrhythmia and death. The elucidation of the responsible mechanisms in the animal models may lead eventually to new and effective interventions to reduce the incidence of sudden death in patients suffering episodes of acute myocardial ischemia.

IV. ISCHEMIA AND INFARCTION

When a coronary artery is occluded in experimental animals, the heart suffers a marked deficiency in oxygen to the areas dependent upon flow through the affected artery. Formerly, it was assumed that

the involved tissue rapidly became ischemic and that only a brief interval elapsed before the damage progressed irreversibly to infarction. The time during which complete occlusion is extant is of particular interest in determining the size of the infarct that eventually develops. Immediately following the occlusion of a coronary artery, there is a period of ischemia that persists for some time. This period is characterized by systolic bulging occurring within several beats after occlusion, depression of systolic shortening, and increased diastolic compliance (Wyatt *et al.*, 1976). Kawamura *et al.* (1976) have studied the length of time (after complete occlusion) necessary in order for an ischemic area to progress to an area of irreversible damage leading to cardiogenic shock. As one might suspect, these authors found the longer occlusion was maintained the larger the area of ischemia and extent of infarction. If the period during which the occlusion was maintained remained brief, then the damage sustained by the myocardium was reversible up to a certain time limit. In this regard, Jennings *et al.* (1975) reported from results obtained in dogs that with a severe degree of ischemia, as would result from almost total occlusion of an artery, the time limit to reach irreversible damage to the myocardium is 20–60 min. There appear to be, therefore, several developmental stages between the time of occlusion and the establishment of an infarct (Jennings *et al.*, 1975; Lie *et al.*, 1975). During this time period of developing infarct there are different periods of arrhythmia.

Recently, the concept of irreversible damage has come under closer scrutiny since it has been shown experimentally that the amount of ischemic tissue that eventually becomes infarcted can be limited through a variety of pharmacologic interventions (Maroko *et al.*, 1972a, b, c; Libby *et al.*, 1973; Epstein *et al.*, 1975). These studies have been performed, for the most part, in dogs and rats, but it is important to point out that similar studies using the pig have not confirmed a beneficial effect for certain pharmacologic agents (Most *et al.*, 1976; Capone and Most, 1979). Toward this end, it is hoped the prognosis following infarction in the clinical setting will improve if the quantity of viable tissue that remains to provide for normal myocardial function is increased. Thus, if the amount of tissue damage could be effectively reduced, the hemodynamic sequelae and death because of pump failure might be significantly reduced. In order to determine which modalities may have utility in the clinical setting, evaluation of these agents in experimental animal models is required. However, before these agents can be evaluated as to whether they alter the amount of tissue that becomes ischemic, reliable techniques must be employed to quantify the amount of ischemic myocardium resulting from coronary artery occlusion.

A. Methods of Quantifying Myocardial Ischemia and Infarction

Attempts to estimate the extent of ischemic injury following coronary artery occlusion have employed either indirect histologic markers to quantify the degree of myocardial perfusion or direct electrophysiological, histologic, or biochemical markers to quantify tissue damage. The indirect estimates of ischemic injury, which involve quantifying myocardial perfusion, have been used to identify the myocardial area at risk, that is, areas of the myocardium receiving no blood flow or very limited blood flow. It is important to appreciate that these indirect methods are unable to discern whether the myocardium is undamaged or damaged, either reversibly or irreversibly (infarcted). On the other hand, the direct estimates of ischemic injury discern whether a region of myocardium is damaged or undamaged, but they do not quantify perfusion. Some direct estimates of ischemic injury can, furthermore, discriminate between damage that is potentially reversible or irreversible. Various indirect and direct methods to estimate the extent of ischemic injury will now be considered.

1. Direct Measurements of Ischemic Injury. Various electrophysiological, biochemical, radionuclide imaging, and histologic markers have been employed to directly measure the extent of ischemic injury to the myocardium after coronary occlusion.

a. Histologic. While electrophysiological and biochemical parameters are useful techniques to determine the amount of tissue involvement following myocardial infarction, they are only estimates of the actual amount of injured tissue. In order to obtain morphological visualization and topographic localization of the infarcted tissue, histologic techniques must be employed. These procedures are the standard against which electrophysiological and biochemical procedures are compared. These methods include both macroscopic and microscopic morphology as well as chemical analysis. Under light microscopy using hemotoxylin and eosin (H&E), initial changes in the morphology of ischemic cardiac cells include widely separated and wavy myofibrils and a loss of stainable glycogen (Jennings and Ganote, 1974). However, even before these intracellular structural changes appear, Hoffstein *et al.* (1975) have demonstrated with colloidal lanthanum that the plasma membrane has suffered ischemic damage resulting in increased permeability to ions and macromolecules. These changes correspond to alteration in the metabolic state of the ischemic tissue, that is, intra-

cellular accumulation of lactic acid, organic phosphate, creatine, hydrogen, and other metabolites. However, these early changes are reversible if flow is restored within approximately 15 min. Longer periods of ischemia result in irreversible damage which is characterized by more severely stretched myofibrils and a complete loss of glycogen (Jennings and Ganote, 1974). With the use of the electron microscope, defects in plasma membranes can be visualized as well as swollen mitochondria (Jennings *et al.*, 1975). These mitochondria also undergo changes as indicated by peripheral aggregation of chromatin material.

Additional histologic studies, using the periodic acid–Schiff reaction (PAS Stain) for glycogen visualization, vital stains for mitochondrial enzyme activity [nicotinamide adenine dinucleotide-diaphorase; NADH-diaphorase and succinic dehydrogenase (SDH)], and additional histologic stains (oil red) for measuring neutral lipid accumulation, have demonstrated in the pig (Janse *et al.*, 1979), dog (Reimer *et al.*, 1977), and rat (Fishbein *et al.*, 1980), that an area intermediate in cellular damage between normal and irreversibly damaged myocardium exists for up to 6 h after the onset of ischemia. This area has been referred to as a "border zone," although there is some disagreement as to whether or not this area in fact exists, especially in the dog (Factor *et al.*, 1978; Marcus *et al.*, 1975). In the rat, this "border zone" is initially characterized after coronary artery occlusion by a depletion in glycogen with near normal enzymatic content. However, with time (1) the enzymatic content of this tissue also becomes depleted in the same areas initially shown to be glycogen deficient and (2) the "border zone" in the rat takes on the appearance of infarcted tissue indicating irreversible change. Thus, specialized histologic stains can be used to demonstrate developing tissue damage in addition to quantifying the total amount of tissue that may become infarcted.

In our laboratory, methylene blue (MB) and nitro blue tetrazolium (NBT) are used to quantify the amount of ischemic and infarcted tissue, respectively, in the cat heart following coronary occlusion. MB is a deep blue dye that becomes colorless when it is reduced to leukomethylene blue by the hydrogen ion excess that accumulates in the ischemic tissue when metabolism is shifted from aerobic to anerobic (Kloner *et al.*, 1975). Thus, normal or aerobic tissue is blue, while anerobic (ischemic) tissue is pink. This makes it possible to quantify the amount of ischemic tissue which develops very rapidly following coronary artery occlusion. The amount of infarcted tissue is quantified with the use of NBT, a dye that acts as a substrate for tissue (especially mitochondrial) dehydrogenase, staining the tissue blue (Nachlas and Shnitka, 1963). Because infarcted tissue does not contain these en-

zymes to any appreciable degree (Wachstein and Meisel, 1955), this tissue appears pale in comparison. We have obtained very reproducible results in cat hearts with this procedure (Ritchie *et al.*, 1979). More recently, using MB, we have identified an ischemic zone of tissue in the cat heart that appears to be intermediate in damage between normal and infarcted tissue. In hearts removed 5 h after abrupt LAD occlusion in cats anesthetized with α-chloralose, staining with NBT indicated that approximately 39% of the ventricles (60% septum, 30% left ventricle) were infarcted, compared to MB treatment, in which approximately 62% of the ventricles were classified as ischemic and/or infarcted. Since approximately 39% of the ventricles represented infarcted tissue (NBT data) this indicated that nearly 23% (62% − 39% = 23%) of the damaged area was still ischemic and probably reversible in nature. Other indices of ischemia were used to verify that MB staining delineated ischemic from nonischemic tissue. Thus, similar to the pig, rat, and dog, an area of tissue remains partially viable up to 5 to 6 h following coronary occlusion in the cat. This "border zone," possibly amenable to pharmacological intervention, exists in the cat after coronary occlusion.

b. Electrophysiologic. Since the initial description of the electrophysiologic changes associated with myocardial infarction by Pardee (1920), numerous studies have been published outlining the usefulness of electrophysiologic techniques to predict the extent of myocardial injury following myocardial infarction (Reid *et al.*, 1971; Maroko *et al.*, 1972a, b, c; Reid *et al.*, 1974; Holland and Brooks, 1975; Heng *et al.*, 1976). Among the first electrophysiological signs of myocardial ischemia are shifts in the S-T segment of the ECG. While certain questions have been raised as to the validity of this technique for predicting the extent of myocardial necrosis (Ross, 1976), the evidence indicates, that, under proper conditions, changes in the S-T segment can be a useful procedure in the evaluation of tissue damage following myocardial infarction. Numerous studies by Maroko and co-workers have demonstrated in dogs that S-T elevation is a reliable marker for the degree of ischemic injury in the left ventricle following occlusion of the left anterior descending coronary artery (Maroko *et al.*, 1971, 1972a, b, c; Maroko and Braunwald, 1973). They evaluated the average changes in the S-T segment occurring in 10 to 14 epicardial leads on the anterior surface of the left ventricle, and found good correlation between the magnitude of S-T segment changes and histologic and biochemical assessments of the degree of tissue injury 24 h after occlusion. Agents found to reduce ischemic damage diminished the S-T segment

elevations while agents known to exacerbate the degree of ischemic injury enhanced the S-T segment elevations. Their biochemical assessment of injury involved measuring the depletion of myocardial creatine phosphokinase (CPK) activity 24 h later. This biochemical parameter correlated well with the S-T segment elevation in the same sampling site 15 min after ligation. These findings were also confirmed histologically. In an additional study, Maroko *et al.* (1972b) demonstrated that the epicardial recordings of S-T segment changes are also in agreement with multiple lead precordial recordings in patients suffering from myocardial infarction. However, Holland and Brooks (1975) found in the pig that multiple lead precordial electrocardiographic S-T segment elevations directly correlated with the size of ischemic injury, while the epicardial ECG S-T segment elevations were inversely related to the size of ischemic injury. Precordial and epicardial ECG S-T segment deflections are complex functions of ischemic geometry and their interpretation with respect to the size and shape of ischemic geometry must be done cautiously. While S-T segment changes appear to be valuable in evaluating the degree of ischemic injury following myocardial infarction, their use should be corroborated with other procedures (biochemical, histologic, etc.) in the particular model in which they are to be used before they can be employed to predict the extent of myocardial ischemia. The S-T segment changes have utility as a noninvasive means of estimating infarct size in the clinical setting.

c. Metabolic. Interference in the pattern of normal myocardial aerobic metabolism because of ischemia results in metabolic changes that can be analyzed biochemically (Brachfeld, 1976). Various metabolic parameters have been utilized to evaluate these changes, including lactate accumulation and glycogen depletion. In addition, serum and tissue levels of enzymes, in particular, lactate dehydrogenase (LDH) (Opie *et al.*, 1973) and creatine phosphokinase (CPK) (Shell *et al.*, 1971), have also been used as measures of the amount of ischemia and/or infarction, resulting from coronary occlusion. The realization that several isoenzymes of these particular enzymes are cardiac specific led to the findings that changes in the plasma levels of particular isoenzymes more accurately indicate myocardial damage than total levels (Cohen *et al.*, 1966; Wagner *et al.*, 1973). In particular, following the discovery of a rapid and easy method for separating CPK into its component isoenzymes, the quantitation of cardiac specific CPK-MB has become the principal biochemical marker in evaluating ischemia and/or infarction (Sobel, 1976).

Plasma levels of CPK are detected first since CPK is released faster

and in larger amounts as compared to other enzymes (Kjekshus, 1976). Depression of myocardial CPK and/or elevation of serum CPK levels have been described in the dog (Shell *et al.*, 1971), pig (Janse *et al.*, 1979), cat (Spath *et al.*, 1979), and rabbit (Kjekshus and Sobel, 1970) following myocardial infarction. These changes have correlated well with other indices of myocardial infarction (i.e., S-T segment elevation, histologic changes, etc.). Because changes in the pattern of tissue depletion and/or serum accumulation of CPK are being used to indicate protection following pharmacologic intervention in myocardial infarction, it is important that these changes reflect preservation of the actual cells affected by ischemia. In this regard, it has been shown that increased oxygen availability to perfused anoxic hearts prior to CPK release results in diminution of the CPK subsequently lost in the effluent; increased oxygen availability to these hearts after CPK release has started does not alter effluent CPK levels. These studies indicate that the start of CPK release reflects the transition from reversible to irreversible damage at the cellular level (Hearse and Humphrey, 1975). However, while this indicator of cellular damage is widely used, measuring plasma CPK accumulation requires multiple sampling and is affected by differences in clearance rates from the myocardium and serum distribution factors (Brachfeld, 1976). Many of these considerations have been examined in an excellent review (Shell and Sobel, 1976).

d. Radionuclide Imaging. In addition to the above mentioned electrophysiologic and biochemical markers, scintillation images of the heart employing radionuclides have been employed to determine the amount of ischemic and/or infarcted tissue following myocardial infarction (Carr *et al.*, 1963; Zaret and Cohen, 1975; Holman, 1976). Following intravenous administration, radioindicators are taken up by either the infarcted or normal myocardium and their distribution is determined by external detecting systems employing scintillation cameras. Scintiphotographic localization of acute myocardial infarction has been achieved using radioactive salts of potassium, rubidium, cesium, and thallium, and a number of technetium-^{99m} complexes, including ^{99m}Tc-tetracycline, ^{99m}Tc-pyrophosphate, and ^{99m}Tc-diphosphonate. Monovalent cations such as potassium and cesium are concentrated in the normal myocardium but not in the infarcted areas; the reverse is true for the technetium-labeled complexes such as tetracycline and pyrophosphate (Sternby, 1979). Because these radiopharmaceuticals are preferentially sequestered by either infarcted or normal myocardium, an infarct can be detected directly and its size determined.

Prior to their clinical use, the majority of experimental studies with radionuclides were performed in dogs (Fink/Bennett *et al.*, 1974; Willerson *et al.*, 1975; Zweiman *et al.*, 1975). For example, radionuclide (^{99m}Tc-tetracycline) administered at the time of coronary occlusion was not preferentially sequestered in either the ischemic or nonischemic tissue when coronary blood flow decreased by 55%. Conversely, ^{99m}Tc-tetracycline became concentrated in acutely infarcted myocardium, but not in nonischemic tissue. Thus, radionuclide concentration correlated with reductions in coronary blood flow. The size of the infarct as determined by scintiphotography correlated well with estimates utilizing serum enzyme activity and electrophysiologic means (Cook *et al.*, 1974).

In patients identification of acute myocardial infarction has been achieved following scintillation imaging of several different radioindicators, including ^{99m}Tc-tetracycline and ^{99m}Tc-pyrophosphate. While these agents are effective in delineating infarct size, their utility in determining the amount of tissue that can recover from ischemic injury is limited. However, among the technetium complexes, tetracycline and glucoheptonate are fairly specific for infarcted tissue while diphosphonate appears to be a more sensitive index for ischemic tissue (Zweiman *et al.*, 1975). Tissue accumulation of diphosphonate appears to be more sensitive to reductions in flow than tetracycline and glucoheptonate accumulation. Accumulation of the tetracycline and glucoheptonate complexes requires a more drastic reduction in flow. Areas where diphosphonate accumulates might recover if appropriate pharmacologic interventions are initiated (Zweiman *et al.*, 1975).

While the above agents, under the proper conditions, can be used to determine infarct size and to a lesser extent ischemia, they do not indicate the actual metabolic state of the underlying tissue. In an attempt to evaluate this state, myocardial metabolic substrates inherent in physiological processes have been labeled with nuclides. The substances employed include [^{14}C]glucose, [^{14}C]octanoate, and [^{14}O]palmitate and various amino acids (Gelbard *et al.*, 1974; Weiss *et al.*, 1975; Ter-Pogossian *et al.*, 1975a). This approach may lead to the development of methods that will monitor the metabolic state of cardiac tissue more directly, as they indicate tissue viability rather than measure the amount of tissue affected.

While radionuclide imaging has been used widely to evaluate acute myocardial infarctions, it has several limitations. In particular, the scintillation camera converts the image of a three-dimensional object into a two-dimensional plane, superimposing the activity in overlying and underlying tissue resulting in noise, artifacts, and decreased con-

trast (Ter-Pogossian, 1976). In addition, the field of view and resolution of a scintillation camera changes with depth, making quantitative measures difficult (Ter-Pogossian, 1976). These problems have been successfully overcome by the discovery of emission computerized axial tomography (CAT). The use of CAT results in transaxial sectional images of organs and structures permitting actual visualization of the gamma-ray-emitting radiolabels (Ter-Pogossian *et al.*, 1975b). The resulting image is not contaminated by label contained in adjacent structures and is depth independent (Ter-Pogossian, 1976). In addition, use of the numerical analog supplied by the online computer allows for precise determination of label concentration in the organ. Successful use of emission CAT has been applied to the myocardium in animal studies (Weiss *et al.*, 1975) and patients (Ter-Pogossian *et al.*, 1975a, b). While it is apparent that this procedure cannot be carried out routinely in the laboratory, its use on a limited basis in combination with labeled metabolic substrates may provide a more accurate assessment of tissue involvement following myocardial infarction.

As was previously mentioned, following myocardial infarction there is an increase in permeability of the myocardial cell membrane, eventually resulting in the release of cellular constituents, including various enzymes and ions. Quantifying this release serves as a means of estimating the degree of cell damage. Khaw *et al.* (1976) proposed that this ischemia-induced increase in cell permeability should also allow the inward diffusion of other macromolecules. Accordingly, it has been proposed that the accumulation of radiolabeled antibody to certain cellular macromolecules might be a means of quantifying the amount of cellular injury following myocardial infarction (Jennings *et al.*, 1975). In particular, cardiac myosin has been used as the intracellular component against which a radiolabeled antibody is directed. Cardiac myosin has several properties that make it useful for this purpose: (1) it is a major intracellular component of the cardiac muscle cell (Katz, 1970) and (2) its high molecular weight (500,000) and solubility properties retard its loss from the damaged myocardium for long periods of time (Jennings *et al.*, 1975). A recent study in dogs has demonstrated that radioactive iodine-labeled antibody specific for canine cardiac myosin was localized selectively and specifically in infarcted tissue (Khaw *et al.*, 1976). In addition, the concentration of radioactive label increased with increasing severity of myocardial damage and showed an inverse, exponential relationship to regional blood flow to the infarcted area. These results indicate that labeled specific antibodies may represent an additional approach to the localization and quantification of myocardial infarcts.

2. Indirect Measurements of Ischemic Injury. Several studies have been directed toward determining the distribution of coronary flow in the left ventricle and the relationship between the reduction of flow and the development of infarction. Myocardial blood flow after acute coronary occlusion has been studied extensively in the dog, an animal with a well-developed epicardial collateral network (Becker *et al.*, 1973; Hearse *et al.*, 1977). Jennings *et al.* (1975) using radiolabeled microspheres quantified regional differences in coronary blood flow and correlated limited perfusion with the histologic severity of ischemia within the same zone following coronary occlusion in the dog. The microsphere technique has recently been found to be unsatisfactory for estimating regions of ischemia in the dog: early expansion of the ischemic area due to edema and cellular infiltration resulted in overestimation of ischemic area (Reimer and Jennings, 1979).

Kloner *et al.* (1975) described an additional method for assessing local changes in flow in relationship to the development of myocardial infarction in an area of ischemia. Utilizing thioflavin S, a fluorescent vital stain for endothelium, these investigators could determine which parts of the capillary bed of the canine left myocardium received blood. The dye was injected intravenously after myocardial infarction prior to sampling. The endothelium of any vessel that received blood containing the dye is revealed by a bright yellow-green fluoresence when illuminated with UV light. Utilizing this procedure, it was determined in the dog that survival of ischemic subepicardial tissue was due to the presence of collaterals as indicated by the stain, whereas the irreversibly damaged subendocardial tissue was due to the absence of any such vessels as indicated by the complete lack of any stain in this area.

One additional indirect measurement of ischemic extent employs coronary arteriography. At the time of sacrifice, the aorta is perfused in a retrograde manner with either a dyed latex solution or a dyed gelatin solution while simultaneously the ligated coronary artery is perfused with a similar solution in a contrasting color. With the aortic and coronary arterial perfusion pressures equivalent, the arterial network supplied by both occluded and nonoccluded arteries should be delineated. This procedure has been used with varying degrees of success in the dog (Rivas *et al.*, 1976; Lowe *et al.*, 1978; Jugdutt *et al.*, 1979).

From the above discussion, it is apparent that there are numerous techniques and/or procedures available to aid in the quantification of ischemic and/or infarcted tissue following myocardial infarction. While each one has its own particular advantages and disadvantages, many have proven to be useful under the appropriate conditions in several different animal species, and in some cases clinically as well.

However, in each case, before a protocol is designed utilizing a particular procedure, its reliability as a marker of damaged tissue should be verified by several other techniques in order to determine that there is good agreement between each of them. These procedures will enable researchers and clinicians alike to evaluate old and new agents in order to find effective therapy in reducing the amount of ischemic and/or infarcted tissue following coronary occlusion.

B. Mechanisms Responsible for the Ischemic to Infarcted Transition

In the previous section we have discussed some of the physiological and/or biochemical changes associated with experimental myocardial infarction, and have evaluated some of the techniques used to quantify the extent of ischemic and/or infarcted tissue that results from occlusion of a major coronary artery. While it may be a desirable goal in the treatment of patients with MI to reduce the amount of tissue that becomes infarcted, the mechanisms responsible for the transition of ischemic tissue to irreversibly damaged infarcted tissue are under investigation in many laboratories, using various animal models. At present, many studies have persuasively argued that cardiac cell death is due to lysosomal enzyme destruction of vital cellular organelles (Fozzard 1976; Lefer *et al.*, 1977). It is difficult to exclude the possibility that an earlier event in the damage process tipped the balance to cell death; lysosomal enzyme activation would then be just an appropriate terminal step. Numerous studies point to cellular calcium ion overload as a possible critical step (Shen and Jennings, 1972a, b; Ichihara *et al.*, 1979; Singal *et al.*, 1979; Weishaar *et al.*, 1979). Yet, in the dog, calcium chelated blood failed to prevent reperfusion induced cell death (Rocco *et al.*, 1979). Most investigators, however, believe that myocardial oxygen supply and demand probably play a role in this tissue transition from damaged to dead (Radvany *et al.*, 1975). Thus, differences in animal myocardial vasculature effect this oxygen supply/demand balance and should alter the degree of ischemic tissue death.

Although the coronary arterial vasculature of the human, pig, and cat are similar (Habermehl, 1959; Schaper, 1971; Cohen, 1979), differences exist between the coronary arterial vasculature of the human, dog, and rat. The coronary arterial vasculature of the dog has a preformed network of intracardiac epicardial arteriolar connections, conventionally called collaterals, that are more extensive than the suben-

docardial distribution of collaterals found in the human heart and in the pig heart (Schaper, 1971). In both the human and the pig, unlike the dog, collateral enlargement as an acute adaptive mechanism is doubtful (Cohen, 1979). Thus, in the dog, experimental infarcts are usually smaller than the perfusion area of the occluded artery (Maroko, 1972a, c; Reimer *et al.*, 1977; Jugdutt *et al.*, 1979) and infarct extent is less predictable than in the pig. The rat has both intracardiac arteriolar collaterals and extracardiac arteriolar collaterals to distinguish its coronary circulation from that of the human or any other animal model considered in this discussion (Halpern, 1957; Bloor *et al.*, 1967). The intracardiac collaterals originate in the atria while extracardiac collaterals originate from the internal mammary arteries. Yet, despite the existence of this collateral network, a peculiar cytoarchitectural difference in the rat myocytes, particularly large mitochondria, makes the rat heart exceptionally vulnerable to infarction (Schaper, 1980). The differences and similarities between coronary arterial vasculature between the human, dog, cat, pig, and rat, as summarized in Table II, should be considered before selecting an animal model to investigate the transition of tissues from ischemic to infarcted.

TABLE II
Comparison of Characteristics of Various Models for Studying Myocardial Infarction

	Dog	Cat	Rat	Pig	Human
Coronary vasculature					
Characterized	Yes	Yes	Yes	Yes	Yes
Predictable pattern	No	Yes	No	Yes	Yes
Extracoronary supplementation	No	No	Yes	No	No
Extensive preformed collaterals	Yes	No	Yes	No	No
Arrhythmia					
Early arrhythmias	Yes	Yes	Yes	Yes	Yes
Brady	Yes	Yes	Unreported	Unreported	Yes
Tachy	Yes	Yes	Yes	Yes	Yes
VF	Yes	Yes	Yes[a]	Yes	Yes
24-Hour arrhythmia	Yes	No[b]	Unknown	Unknown	Variable
Late arrhythmia (3–7 day)	Yes	No[b]	Unknown	Unknown	Variable
Ischemia/infarction					
Predictable incidence	No	Yes	Yes	Yes	Yes
Predictable infarct extent	No	Yes	Yes	Yes	Yes
"Border zone"	Variable	Yes	Yes	Yes	Yes
Acceptance by scientists	Good	Unknown	Gaining	Good	Standard

[a] Reversible.
[b] Except with A–V node sectioned.

V. SUMMARY

In summary, the use of animal models to study the sequelae of coronary artery occlusion must be approached with an appreciation of the advantages and disadvantages of each species. Although the dog has been used traditionally for many of these studies, investigators should not be reluctant to use other species when a clear advantage for that species is known to exist. One major advantage of the dogs is that extensive literature exists, allowing for comparisons. Yet, the availability of a species with known genetic characteristics, age, weight, range, and coronary vasculature may outweigh this advantage. We have attempted to summarize some of the factors that we feel are important in selecting an appropriate animal model for studying myocardial ischemia and infarction (Table II).

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3 Pharmacologic Approaches to the Prevention of Myocardial Infarction and Sudden Death

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Myocardial Infarction and Cardiac Death

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I. INTRODUCTION

Sudden death from ischemic heart disease remains one of the major public health problems in the United States. The medical treatment for primary and secondary prevention of sudden death and myocardial infarction will be reviewed in this chapter. Pharmacologic interventions can be considered under two broad headings. The first is the chronic administration of drugs directed at prevention of sudden death in individuals who have high risk; the pharmacologic agents are aimed at suppressing lethal factors (arrhythmia, hypertension, hyperlipedemia, coaguability). The second is the administration of an active agent that may interfere rapidly with either the metabolic or electrophysiological phenomenon occurring with the acute cardiac event.

The long-term medical approach to therapy and the prevention of sudden death in patients with ischemic heart disease requires identification of those patients who were at the highest risk of developing sudden cardiovascular collapse. Many prognostic indices have been developed to identify these high-risk individuals based on the patient's age, a history of diabetes mellitus, myocardial infarction, and angina pectoris. Other factors include internal pressure, clinical evidence of left ventricular function, and the presence of arrhythmia (Braunwald, 1980). The current emphasis in therapy is on the use of antiarrhythmic agents in those patients who have demonstrated increased frequency of ventricular premature contractions with the assumption that these arrhythmias have relationships to sudden cardiac death. Additional approaches to the long-term prevention of sudden death have included the use of anticoagulants (antiplatelet drugs and sodium warfarin) and the manipulation of various risk factors (hypertension, lipids) as secondary preventive measures in ischemic heart disease.

A more urgent public health issue is administration of various pharmacologic agents directed at the treatment of the acute cardiac event. This approach to therapy is based on the assumption that there are certain patients with acute chest pain in whom the rapid administration of a pharmacologic agent could prevent sudden death. Studies by a number of investigators have raised important questions as to how frequently an acute prodromata precedes sudden death. Nevertheless, there does appear to be a period of time where the death of some individuals could be prevented by a medical intervention. The time span here is minutes and therefore too short for considering the use of anticoagulants or longer-acting antiarrhythmic agents having onsets of action greater than an hour or two. The issue here deals primarily with

the question of administering antiarrhythmic agents or coronary vasodilators that have a rapid onset of action. There are individuals who will die instantly and those who die within the first few minutes to 1 h from the onset of symptoms. It is for this group of patients that administration of drugs may be effective in preventing sudden death. The main limitation of this type of medication is that it must be self-administered, thus excluding most parenteral medications (Goldstein, 1974).

In this chapter some of the newer pharmacologic approaches to the prevention of sudden death will be reviewed, including anticoagulation, antiarrhythmic prophylaxis, β–adrenoceptor blocking drugs, lipid-lowering interventions, and the utilization of vitamin E.

II. β-ADRENOCEPTOR BLOCKING DRUGS

β-Adrenoceptor blocking drugs have been shown to be safe and effective in the treatment of patients who have hypertension, angina pectoris, hypertrophic cardiomyopathy, thyrotoxicosis, glaucoma, and migraine headaches (Frishman and Silverman, 1979b). However, the role of these agents in the treatment and prevention of myocardial infarction is controversial due to a lack of adequately controlled clinical trials.

These agents are employed with a view toward improving the quality as well as the quantity of life, even though there is no adequate clinical data to prove the latter. The theoretical considerations and clinical experience in the use of β-adrenoceptor blocking drugs for the primary and secondary prevention of acute myocardial infarction and sudden death are assessed below.

A. Primary Prevention (Myocardial Infarction and Sudden Death)

Whereas β-adrenoceptor blockade does not directly affect the progress of coronary atheroma, it does reduce myocardial oxygen requirements (Conolly *et al.*, 1976). This in turn enables the cardiac muscle to tolerate, for a while, what would otherwise be an inadequate supply of oxygen (Conolly *et al.*, 1976). The reduction of oxygen requirements is due to a decreased heart rate and reduction in myocardial contractility

(Epstein *et al.*, 1965). β-Blockade may improve distribution of flow in the ischemic myocardium (Becker *et al.*, 1971) as well as improve the utilization of myocardial substrate (Opie and Thomas, 1976). These drugs are known to inhibit platelet aggregation (Frishman *et al.*, 1974) and to be potent antiarrhythmic agents (Jewitt and Singh, 1974).

In patients with angina pectoris, these agents have been useful in reducing pain and improving exercise tolerance. (Prichard and Gillam, 1971). An adequate degree of β-adrenoceptor blockade affords marked relief from pain in 80 percent of the cases. This success rate can be improved by selecting the best-tolerated β-blocker in combination with nitrates (Wiener *et al.*, 1969). Thus, the quality of life can be improved in patients with angina pectoris.

A major unresolved question in clinical medicine is whether the β-adrenoceptor blockers can influence the natural course of stable angina pectoris by prolonging the patient's life expectancy and by reducing the risk of infarction. There are multiple subgroups of patients with angina pectoris defined by differences in symptomatology (stable or unstable), coronary anatomy, left ventricular function, and stress test results. These multiple variables make a proper study design almost untenable. The few poorly designed studies that have been completed have not demonstrated any marked reduction in the annual mortality rate of patients with stable angina pectoris when compared to results of large epidemiologic studies (Amsterdam *et al.*, 1968; Lambert, 1975; Russek, 1975; Warren *et al.*, 1976; Kannel and Feinlab, 1972). While patients are treated with β-adrenoceptor blockers to increase the quality and quantity of life, only the quality of life has proven to be enhanced.

Nevertheless, β-blockade is the treatment of choice for the symptomatic relief of angina pectoris. However, as in the case with lipid-lowering and platelet-inhibiting agents, only theoretical considerations suggest employing β-adrenoceptor blocking drugs as primary prophylactic treatment against myocardial infarction and sudden death. Prospective studies with high-risk individuals are necessary to determine whether primary preventive treatment will indeed prolong life and/or prevent sudden death. There is some suggestive data however. In a study of over 1000 middle-aged hypertensive males, a reduction of approximately 50 percent in the incidence of fatal and nonfatal myocardial infarction was most likely attributed to the treatment with β-adrenoceptor blocking drugs (Berglund, *et al.*, 1978). In lieu of definitive proof of efficacy, at this juncture primary prophylactic treatment is cost ineffective.

B. β-Adrenoceptor Blocker Therapy in Acute Myocardial Infarction

In the treatment of acute myocardial infarction, the effectiveness of β-adrenoceptor blockade depends upon the functional state of the heart. The more left ventricular function is determined by the mechanical and contractile properties of ischemic areas, the more likely it is that β-adrenoceptor blockade will improve oxygenation of jeopardized myocardium. With careful hemodynamic monitoring of patients, therapeutic benefits and potential hazards can be assessed. The early use of β-adrenoceptor blockade may interrupt the stepwise development of myocardial necrosis, may salvage myocardial tissue, and may improve immediate mortality rate and long-term ventricular function (Frishman and Sonnenblick, 1977; Frishman, 1979).

C. Secondary Prevention of Myocardial Infarction and Sudden Death

There is currently a 10% mortality rate 1 yr following a myocardial infarction (Pell and d'Alonzo, 1964; Norris and Mercier, 1973) and this mortality occurs within 1 h of the onset of symptoms in approximately half of these cases. A therapeutic goal is to decrease the mortality rate in this group of patients who are at high risk of sudden death. Ventricular tachyarrhythmias are now considered the most important cause of sudden death in patients who are less than 70 yr old.

A significant reduction in mortality by continuous treatment with procaine amide, quinidine, phenytoin, as well as other new antiarrhythmic agents after myocardial infarction has not been demonstrated in controlled clinical trials (Kosowsky *et al.*, 1973; Collaborative Group, 1977). Short-term trials using propranolol following acute myocardial infarction have shown conflicting results. Snow (1965) first reported a substantial reduction of mortality in patients with myocardial infarction who had received propranolol. He treated 52 patients with 60 mg of propranolol daily for 21 days and compared them to 55 control subjects. The results, while impressive, were not conclusive because it was an open label study without a double-blind control

group. Several randomized double-blind studies were than initiated. (Balcon *et al.*, 1966; Clausen *et al.*, 1966; Norris *et al.*, 1968; Stephen, 1966). These included about 250 patients with acute myocardial infarction who received an average of 40 to 80 mg of oral propranolol daily. These controlled studies failed to confirm the findings of Snow. There were no significant differences in mortality, shock, heart failure, hypotension, or arrhythmias between the treated and the untreated patients.

The first long-term study using a β-adrenoceptor blocker also failed to demonstrate a decreased mortality rate. (Reynolds and Whitlock, 1972). In this double-blind randomized study, 78 patients were given 100 mg of alprenolol or placebo four times daily, for 1 yr, following the time of admission to a hospital coronary care unit. This trial may have been of too short a duration and may have involved too few patients to show a difference.

Two major clinical trials from Sweden (Wilhelmsson *et al.*, 1974; Ahlmark *et al.*, 1974) and one from Denmark (Andersen *et al.*, 1979) using alprenolol in patients with myocardial infarction have since been completed. Alprenolol was chosen as the β-adrenoceptor blocker for study owing to its moderate intrinsic stimulating action and consequent theoretically reduced risk of causing sinus bradycardia. The objective of these studies was to investigate whether the incidence of recurrence of infarction, sudden death, and total mortality was influenced in patients treated with a β-adrenoceptor blocker.

In the study by Wilhelmsson *et al.* (1974), patients who had a myocardial infarction were less prone to sudden death if treated with a β-blocking drug. In this small but well-controlled study, 230 patients who had sustained a myocardial infarction were grouped into four separate risk strata which were determined by the degree of previous myocardial damage. Within each stratum alprenolol (400 mg daily) or placebo was randomly assigned to patients over 2 yr. Within each stratum, the incidence of nonfatal reinfarction appeared much the same, but the incidence of sudden death and total mortality was significantly reduced in patients receiving alprenolol. These results have been questioned because of the use of a fixed-dose regimen and the small sample size analyzed.

In the study by Ahlmark *et al.* (1974), a similar reduction in sudden death was seen in patients treated with alprenolol compared to matched controls. In contrast to the findings of Wilhelmsson and colleagues, these investigators also reported a reduction in the actual reinfarction rate in patients receiving alprenolol. This single-blind ran-

domized trial was carried out for 2 yr in 162 patients who had sustained a myocardial infarction. Patients received either 100 mg of alprenolol four times daily or no β-blocker. This study has been criticized on the grounds that the patients were randomized before their eligibility for the study was determined and because the study was open and the control group was not given placebo.

Finally, in 1979, a Danish trial was published (Andersen *et al.*, 1979) that supported the conclusions of the two previous studies. In this controlled study, patients with acute myocardial infarction received either placebo or 400 mg of alprenolol a day. Intravenous alprenolol was started as soon as possible following admission to the hospital and was continued orally (400 mg/day) for 12 months. It was noted that β-adrenoceptor blockade was beneficial in postinfarction patients under the age of 65 yr but of no benefit in older patients.

The largest European trial of a β-adrenoceptor blocker to date in patients with myocardial infarction was a double-blind randomized Multicenter International study (1976) in over 3000 patients comparing practolol with placebo. One to four weeks after an acute myocardial infarction, fixed-dose practolol (200 mg twice daily) or placebo was randomly assigned to patients. Unfortunately, the trial was prematurely terminated after the reports of practolol toxicity emerged. However, 330 actively treated patients and 336 treated with placebo were followed for over 2 yr. A significant difference was found in the incidence of sudden death within 2 h after the onset of symptoms—30 patients in the practolol group compared with 52 patients in the placebo group. There was also a significant difference in the total number of cardiac deaths. A nonsignificant trend toward reduction in nonfatal reinfarctions, 69 compared with 89, was also found. In this study, the beneficial effects seem to be confined to patients with previous anterior wall myocardial infarcts and low diastolic blood pressures at entry. The study also showed a significant reduction in cardiac arrhythmias and in the incidence of angina pectoris with practolol.

Three recently reported clinical trials provide convincing evidence that both sudden cardiac death and reinfarction may be prevented in survivors of an acute myocardial infarction. The results of the Norwegian Multicenter Group Study (1981) utilizing timolol in doses of 10 mg b.i.d. provides convincing evidence that both reinfarction and sudden death are decreased in patients of all ages, regardless of the infarction site, when treatment began 7–28 days after an MI and continued for a period averaging 17 months. One hundred fifty-two deaths oc-

curred in the 939 patients on placebo; 98 in the 945 patients in the timolol group. Cumulative reinfarction rates were 20.1% in the placebo group; 14.4% in the timolol group. After 6 months, however, reinfarction rates were the same.

In the American study, β-Blocker Heart Attack Trial Research Group 1982 (BHAT) which used propranolol in dosages of 180 to 240 mg/day in three divided doses, patients were started on therapy 5–21 days after an infarction. More than 3800 patients participated in this study, which was recently discontinued because of a significant reduction in mortality of over 25% in the propranolol group as compared with a placebo group over an average period of 2 yr. Overall mortality was 9.5% in the controls and 7% in the treated group. Most of the benefit occurred during the first year of treatment.

In another recent study, metoprolol was given intravenously during the first few hours following a suspected or proven myocardial infarction, followed by oral β-blocker therapy over a 3-month period. A placebo comparison group was also followed. A definite reduction in overall mortality in the treated group was demonstrated during the 3-month period (Hjalmarson et et *al.*, 1981). Yusuf et *al.* (1980) have also shown that intravenous administration of atenolol within 12 h of the onset of symptoms of myocardial ischemia reduced mortality and both the frequency and size of the ensuing myocardial infarction.

The evidence in favor of a prophylactic or "cardioprotective" effect of β-adrenoceptor blocking drugs in the management of patients following myocardial infarction is persuasive. At least three possible mechanisms of action might be implicated. Chronic β-adrenoceptor blockade might prevent myocardial infarction (1) by reducing myocardial oxygen demands (Epstein et *al.*, 1965) and/or altering abnormal platelet aggregability (Frishman et *al.*, 1974), (2) by preventing malignant arrhythmias during the initial phase of an infarction (Jewitt and Singh, 1974), and (3) by influencing the size of the ischemic area apart from the antiarrhythmic action of the β-blocker, irrespective of whether infarction occurred. (Mueller et *al.*, 1974).

Would one β-adrenoceptor blocking agent provide a theoretical advantage over another in patients with acute myocardial infarction? With equal degrees of β-adrenoceptor blockade, all the available agents are equally effective in the therapy of hypertension, arrhythmias, and angina pectoris (Frishman and Silverman, 1979a, b). Alprenolol, a noncardioselective agent with membrane stabilizing activity and intrinsic agonist effects (Frishman, 1979), and practolol, an agent that has cardioselective and agonist activities without membrane depressant effects (Frishman, 1979), both appear useful as "cardioprotective"

agents. Therefore it would appear that, if β-adrenoceptor blockade proved to be an effective treatment modality in patients with myocardial infarction, all β-blocking drugs could be interchanged with the exception of small differences in side effects.

D. Conclusion

The results of experimental and clinical research suggest that β-adrenoceptor blockade may play an important role in the prevention of acute myocardial infarction. These β-blockers decrease cardiac work and improve myocardial metabolism. These drugs also are effective antiarrhythmic agents.

β-Adrenoceptor blockers significantly reduce the symptoms of angina pectoris, but have not been shown to influence the life span of patients in primary prevention trials. Following acute myocardial infarction, however, there is some evidence suggesting that chronic β-adrenoceptor blocker therapy can reduce the incidence of sudden death and reinfarction.

Recent studies have determined that β-adrenergic blocking drugs can enhance the quantity of life as well as the quality of life in many patients who have sustained a myocardial infarction.

III. LIPID-LOWERING AGENTS

A. Dietary Effects

Elevated blood lipids have been associated with an increased risk of sudden death and myocardial infarction. It would appear that a reduction in these lipids would reduce the risk of both myocardial infarction and sudden death. Authorities on coronary heart disease differ on whether the general public should be advised to follow a diet that is low in cholesterol and saturated fats (Ahrens, 1979; Blackburn, 1980). Many authorities agree, however, that the risk of coronary disease is lower in patients with low serum cholesterol levels; therefore, the best advice for patients with an elevated serum cholesterol concentration is to reduce it by diet or, if diet fails, by taking lipid-lowering drugs.

1. CLOFIBRATE (ATROMID-S). The serum lipid fraction now most firmly associated with coronary artery disease is cholesterol. Serum triglycerides are no longer considered as important (Hulley *et al.*, 1980). Nevertheless, the best-studied and probably most widely used lipid-lowering drug is clofibrate, which is most effective in lowering serum triglycerides, but less effective than several other agents in lowering cholesterol. Clofibrate has also been shown to be an effective inhibitor of platelet aggregation (Glynn *et al.*, 1967).

Several clinical studies documenting the efficacy of clofibrate in preventing death in patients with coronary artery disease have been reported in recent years. A cooperative study performed in Newcastle-on-Tyne (1971), demonstrated a significant reduction in the incidence of death in patients with evidence of angina pectoris or myocardial infarction, or both, who received treatment with clofibrate and were followed up over a 5-yr period. The beneficial effect of the drug in 497 patients randomly assigned did not appear to correlate with a specific effect of the drug on blood lipid levels. A similar study performed by the research committee of the Scottish Society of Physicians (1971) found similar results in patients followed up for 6 yr, although they noted a significant decrease in death rate only in their patients who had angina pectoris with or without a previous infarction and not in those who had a previous infarction but no angina. Again the beneficial effect of the drug did not appear to be related to the lipid-lowering effect.

A randomized study performed in United Airlines personnel (Krasno and Kidera, 1972) demonstrated a significant reduction in the occurrence of myocardial infarction among men initially free of evidence of coronary artery disease and among men who had a history of myocardial infarction or angina, or both, who were randomized to treatment with clofibrate. These findings were unrelated to any lipid-lowering effect noted. The lack of correlation of benefit with serum lipid effects suggests that another mechanism, such as the platelet aggregation inhibition known to occur with the drug, might be operating. However, the Coronary Drug Project study (1975) of clofibrate did not confirm the findings in these three studies. There was no significant benefit with regard to mortality noted among the patients with a history of at least one myocardial infarction who have been randomized to treatment with clofibrate. The reason for this discrepancy remains unclear but probably relates to patient selection. The positive British studies included patients with angina alone, suggesting that possibly clofibrate is of greater benefit when begun early in the course of the disease.

A double-blind study conducted by the World Health Organization (1978) compared clofibrate with placebo in 10,000 men in the upper

third of the distribution of serum cholesterol levels. The results demonstrated a decrease in nonfatal myocardial infarction in those treated with clofibrate, but no decrease in fatal myocardial infarctions. Clofibrate-treated patients had a higher noncardiac mortality rate than controls, due mainly to an increased incidence of malignant neoplasms and gastrointestinal problems, including complications of cholecystectomy.

These recent data support the conclusions of the earlier British studies and United Airlines study that clofibrate is effective in decreasing coronary artery disease and morbidity, although they disagree with previous findings in demonstrating that this effect is largely related to, rather than independent of, a cholesterol-lowering effect. Whether the platelet effects of clofibrate are of importance in this cooperative study is conjectural at this time.

2. CHOLESTYRAMINE (QUESTRAN ®) AND COLESTIPOL (COLESTID®). The bile acid binding resins cholestyramine and colestipol are much more efficacious than clofibrate in lowering elevated serum cholesterol levels (Nies, 1978). They usually reduce cholesterol concentrations by 20 to 30% but they are less well tolerated. These resins can cause severe constipation, particularly in older patients, as well as nausea, vomiting, flatulence, and diarrhea. They can interfere with gastrointestinal absorption of other drugs, including chlorthiazide, digoxin, and thyroxine. The cholesterol-lowering effect of the resins can be increased with concurrent use of a second cholesterol-lowering drug, such as niacin or neomycin.

3. NIACIN (NICOTINE ACID). Niacin lowered serum cholesterol concentrations and the incidence of nonfatal myocardial infarction in The Coronary Drug Project (1975). Patients treated with niacin were similar to controls in death due to coronary heart disease, but cardiac arrhythmias occurred more frequently with niacin. Many patients refuse to take niacin because it can cause severe flushing, pruritus, and gastrointestinal distress. It may also cause hyperpigmentation, glucose intolerance, hyperurcemia, and liver toxicity.

4. NEOMYCIN. Another cholesterol-lowering drug that can be used concurrently with cholestyramine or colestipol, or with clofibrate, is the "nonabsorbable" aminoglycoside antibiotic, neomycin (Nies, 1978). The antibiotic dose of 0.5 to 2 g daily has been reported to reduce serum cholesterol levels by an average of 20 to 30% and it has generally

been well tolerated; given with cholestamine or clofibrate, the reduction in serum cholesterol is close to 40%. However, neomycin is actually absorbed in small amounts and it can cause nephrotoxicity and permanent nerve deafness in patients with impaired hepatic or renal function or inflammatory bowel disease.

5. PROBUCOL (LORELCO®). The newest drug marketed in the United States for lowering elevated serum cholesterol levels has not been adequately studied. It is slowly released from fat stores for months after patients have stopped taking it, and its long-term effects are not known (Medical Letter, 1980).

B. Conclusion

Even though the effectiveness of lipid-lowering drugs in reducing mortality from coronary artery disease remains unproven, many authorities recommend their use for patients with elevated serum cholesterol levels that do not respond to diet. A combination of cholestyramine with niacin or neomycin may be the most effective regimen for this purpose. Under pressure from the United States Food and Drug Administration, the manufacturer of clofibrate has recently published advertisements and sent all physicians in the United States a package of printed material to suggest that the use of clofibrate should be limited to patients with elevated plasma lipids who have failed to respond adequately to diet, and who cannot take or fail to respond to other lipid-lowering drugs, and who are at a high risk of coronary artery disease because of other risk factors.

IV. VITAMIN E

Vitamin E is one of the four fat-soluble vitamins required by animals for normal cell differentiation and function. Unlike the water-soluble vitamins of the B-complex, these lipid vitamins are not required by microorganisms and most living forms but express their physiological function from higher vertebrates.

The mode of action of vitamin E at the molecular level is not known with certainty. At present, there are three hypotheses (Olson, 1973)

which are under continuing investigation. These are (1) the antioxident hypothesis, (2) the respiratory chain hypothesis, and (3) the genetic regulation hypothesis.

Although vitamin E deficiency in ruminants results in conspicuous heart disease, a similar deficiency state in primates appears to spare the heart, even when other systems are effected. No heart disease in man has been related to a vitamin E deficiency (Olson *et al.*, 1973). The pharmacological use of vitamin E in doses 10–50 times the daily requirement has been recommended since 1947 for the treatment of a variety of cardiovascular disorders, including intermittent claudication, angina pectoris, coronary occlusion, congestive heart failure, thrombophlebitis, and thromboembolism, but no evidence of its effectiveness has been convincingly verified during the ensuing 30 yr (Olson *et al.*, 1973).

V. ANTICOAGULATION THERAPY (HEPARIN AND SODIUM WARFARIN)

A. Methodologies

The role of long-term anticoagulant therapy in prevention of acute myocardial infarction and sudden death has been one associated with controversy since its introduction as a mode of therapy in cardiovascular disease (Frishman and Ribner, 1979). The rationale for suggesting long-term anticoagulation (sodium warfarin) for several years after myocardial infarction as the means of preventing recurrence rests on the assumption that thrombosis is an integral part of the atherosclerotic process, and that the sudden formation of a clot in a major coronary artery is a precipitating event in acute myocardial infarction (Mustard and Packham, 1975; Roberts, 1977). Neither hypothesis has been validated conclusively. The nature of atherogenesis is still a hotly debated issue, and the role of thrombosis is unproved. Moreover, work by Roberts (1977) strongly suggests that the thrombi do not incite infarction but form when necrosis has already taken place. Erhardt *et al.* (1973) injected ^{125}I-labeled fibrinogen intravenously in seven patients soon after admission for acute myocardial infarction. At postmortem study, thrombi in the coronary arteries were found to have diffusely accumulated radioactivity, implying that clotting had followed rather

than proceeded the ischemic event. The validity of this conclusion has been challenged by Moschos et *al.* (1976).

Many clinical studies have investigated the effect of long-term anticoagulation on recurrent myocardial infarction and mortality (Aspentrom and Korsan-Bengtsen, 1964; Loeliger et *al.*, 1967; Meuwissen et *al.*, 1969; Ebert et *al.*, 1969; VA Cooperative, 1973). Most studies have shown a slight decrease in the rate of recurrence of infarction, barely achieving statistical significance. There seems to be some value to anticoagulation in improving survival after myocardial infarction, but this benefit is restricted to men with a history of prolonged angina or previous episodes of cardiac necrosis (Collaborative, 1970). Moreover, the advantage does not appear to persist beyond 2 to 3 yr after initiation of therapy (Hilden et *al.*, 1961; Ebert, 1972). In addition, a large proportion of potential recipients of treatment (up to 50% in some cases) were excluded from randomization because of advanced age or predisposition to bleeding. The methodologies of the long-term studies with anticoagulation have been analyzed by Gross et *al.* (1972). In their review of published reports, no adequate experimental protocol was found.

In contrast to long-term anticoagulation there have been many studies suggesting beneficial effects of anticoagulation (heparin and sodium warfarin) during the acute phases of myocardial infarction for preventing venoembolic complications and peripheral emboli (Frishman and Ribner, 1979). However, in the acute setting, there are few data to support any influence by heparin or sodium warfarin anticoagulation on the extent of myocardial necrosis or recurrence of myocardial infarction (Frishman and Ribner, 1979).

In the British Medical Research Council study (1969), the in-hospital reinfarction rate for subjects given heparin and phenindione was not significantly different from that of the group receiving phenindione alone. These results were supported by the work of Drapkin and Merskey (1972). Similar findings were documented in the Veterans Administration Cooperative Trial (1973), in which both the short-term incidence of recurrent myocardial infarction and the frequency of deaths from reinfarction were essentially equal in the study populations.

The best-designed perspective studies available to date have demonstrated no improvement in the short-term death rates of patients with anticoagulation after myocardial infarction. Two recent retrospective trials have rekindled the controversy, showing a reduction in mortality in hospitalized patients, but a firm conclusion remains elusive (Modan et *al.*, 1975; Tonascia et *al.*, 1975). Wessler et *al.* (1974) calculated that, because of the smaller incidence of deaths from embolism in myocar-

dial infarction, a sample population of 38,000 would be required to demonstrate a significantly beneficial effect of anticoagulation on total mortality. It seems reasonable to suppose that this study will not be undertaken.

B. Conclusion

The potential advantages of anticoagulation in acute myocardial infarction would include (1) prevention of thrombosis in the deep veins of the legs, (2) reduction in the incidence of embolization from cardiac mural thrombi to the brain, kidneys, and limbs, (3) prophylaxis against extension of myocardial necrosis and long-term recurrence of infarction, and (4) reduction of overall mortality related to myocardial infarction. During the acute phase of myocardial infarction anticoagulation (heparin and sodium warfarin) has been shown to be effective in reducing the incidence of venoembolic and arterial embolic complications of acute myocardial infarction. The incidence of mortality from pulmonary emboli as a complication of myocardial infarction is probably influenced by anticoagulant treatment. However there is no data to suggest that during the acute phase of myocardial infarction anticoagulation will prevent extensive myocardial necrosis or recurrence of myocardial infarction. There is certainly no data to advise the use of anticoagulation with sodium warfarin beyond the inpatient phase of myocardial infarction.

VI. ANTICOAGULATION (ANTIPLATELET AGENTS)

A. Methodologies

There is growing evidence to suggest that blood platelets may be playing an important role in symptomatic coronary artery disease (Haft, 1974; Frishman et *al.*, 1974). Evidence is available that platelet aggregates forming inappropriately in the vasculature of the heart can cause ischemic damage. Platelet aggregates can stimulate spasm and may be an initiating factor in the origin of the arteriosclerotic lesion (Haft, 1974). Most of the coronary risk factors in patients have been found to

be associated with platelet function abnormalities (Haft, 1974). Findings such as these have provided the stimulus for the performance of a number of clinical studies that have explored the value of various platelet aggregation inhibiting drugs in the prevention of infarction or death in patients with coronary artery disease. The results of these studies will be described below.

Pathological findings in patients who have died suddenly have supported the concept that an occluding platelet aggregate might be the event that triggers a fatal arrhythmia. Haerem (1974), reported a significantly greater incidence of platelet microthrombi in patients with coronary artery disease who died suddenly than in patients who died from other causes. Forty-seven percent of 47 patients who died suddenly were found to have acute microthrombi in their coronary vessels compared with 15 of 30 patients who did not die suddenly and had no coronary artery disease.

1. Aspirin. There is evidence that regular administration of aspirin may be a benefit to patients with known coronary heart disease. This data has come from several sources. The Boston Collaborative Drug study (1974) compared the previous use of aspirin in hospitalized patients with a discharge diagnosis of myocardial infarction and in patients with other diagnoses. These results show a considerably lower use of aspirin in the myocardial infarction group relative to the other group. A prospective randomized double-blind trial by Elwood *et al.* (1974) compared men with a recent myocardial infarction who were prescribed aspirin (300 mg/day) or placebo. This trial showed an overall trend in favor of aspirin on mortality, but results were not statistically significant. In the Coronary Drug Project aspirin study (1976), also a randomized double-blind trial,

Coronary Drug Project aspirin study (1976), also a randomized double-blind trial, aspirin 972 mg/day or placebo was given to men who survived one or more myocardial infarctions. The mortality in the aspirin-treated group was 30% lower than the placebo group; however, these results did not read statistical significance.

In addition to the previously-mentioned clinical studies, there is considerable evidence that platelet aggregation and platelet-induced thrombosis may play a role in many clinical events associated with coronary artery heart disease. Aspirin can inhibit the information of prostaglandin endoperoxides and thromboxane A_2, substances that aggregate platelets and induce vasospasm (Marx, 1977). However the clinical pharmacology of the drug is further complicated by recent findings that it also blocks the synthesis of prostacyclin (PGI_2), an

inhibitor of platelet aggregation and a vasodilator that is produced in blood vessels (Lipson *et al.*, 1978). The effects of aspirin on platelet aggregation are shown in Fig. 1.

The three early randomized control trials provided suggestive evidence in support of using aspirin as a prophylaxis in coronary heart disease. These led to three more trials that have recently been published: a trial sponsored by the Medical Research Council Epidemiology Unit of South Wales (MRC) (Elwood and Sweetman, 1979), the Aspirin Myocardial Infarction study (AMIS) sponsored by the National Heart and Lung Institute of the National Institutes of Health (1980), and the Persantine-Aspirin Reinfarction study (PARIS) sponsored by a private pharmaceutical company (1980). As initially reported, these studies have still not provided clear-cut proof as to the virtue of using aspirin in a postmyocardial infarction patient. The results still tend to favor aspirin use after myocardial infarction but falls tantalizingly short of statistical significance.

The MRC study (Elwood and Sweetman, 1979) included 1682 myocardial infarction survivors who were randomized into aspirin and placebo groups. After 1 yr, respective mortalities of 12.3 and 14.8% suggested a 17.3 reduction in mortality by aspirin use, which was not statistically significant. When only coronary heart disease deaths were considered, there was statistically significant difference; this was lost when a correction was made for the greater age at entry of the placebo group. However, the nonfatal recurrent myocardial infarction rate was

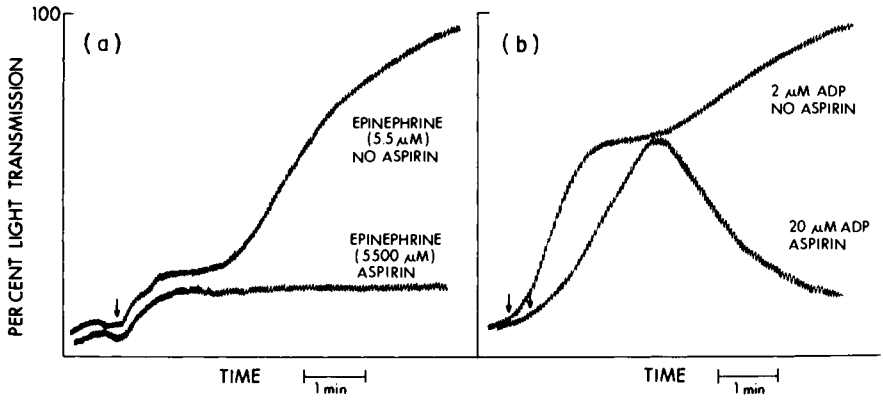

Fig. 1. Effects of aspirin on (a) epinephrine- and (b) ADP-induced platelet aggregation in a patient with angina pectoris. (a) Before aspirin, 5.5 μ*M* of epinephrine produce irreversible aggregation; after aspirin, a thousandfold increase in epinephrine concentration does not induce aggregation. (b) Similar results with ADP as the aggregating agent before and after aspirin. Reprinted from W. H. Frishman et *al.* (1976). *Am. Heart J.* **92**:1 with permission.

significantly reduced in the aspirin group (7.1%) compared with that of the control group (10.9%), as was the combined sum of total mortality plus nonfatal coronary heart disease morbidity. The overall reduction of coronary heart disease incidence, fatal and nonfatal, by aspirin therapy was 28%.

The AMIS study (1980) similarly randomized 4524 postinfarction patients into two groups, the aspirin being given at about the same dose (1.0 g), with perhaps a better level of compliance (88%) than in the MRC study. After 3 yr, death rates were not significantly different in the aspirin and placebo groups. However, again there was a statistically significant difference in the rate of definite recurrent nonfatal myocardial infarctions (22%) and strokes (40%). When deaths from coronary artery disease and definite nonfatal myocardial infarction were combined, the aspirin group (14.1%) was not significantly different than the placebo group (14.8%). Symptoms suggestive of peptic ulcer, gastritis, or erosion of gastric mucosa occurred in 23.7% of the aspirin group and 14.9% in the placebo group.

Both of these studies were flawed by the fact that more patients in the aspirin group had evidence on entry for progressive congestive heart failure (cardiomegaly and digitalis medication). In the AMIS study, there were also more patients with cardiac arrhythmias (antiarrhythmic medication) or angina pectoris in that group than were found in the placebo group. These differences reached statistical significance in each of these patient categories in the AMIS study and may well explain why the anticipated improvement in mortality was not seen. The authors advise that statistical corrections or exclusions to examine these group differences did not alter their conclusions. However, if only a fraction of the excess patients with initial congestive heart failure, angina pectoris, or arrhythmia contributed to mortality in the aspirin group, this could easily have masked an otherwise significant difference in fatal clinical episodes between the two groups (Jones, 1980).

In the Persantine-Aspirin Reinfarction study (PARIS trial, 1980), 2026 patients who had recovered from myocardial infarction were randomized into three groups: persantine plus aspirin ($N = 810$), aspirin alone ($N = 18$), and placebo ($N = 406$). The average length of the follow-up study was 41 months. Results for three specified primary end-points were (1) total mortality was 16% lower in the persantine plus aspirin group and 18% lower in the aspirin group compared with placebo; (2) coronary mortality 24 and 21% lower, and (3) incidence of nonfatal myocardial infarction plus fatal coronary artery disease 25 and 24% lower. These differences were not statistically significant by study

criteria. By life-table analysis, the rates of coronary mortality and coronary incidence were both 50% lower in the persantine plus aspirin group than in the placebo group from 8 to 24 months. Aspirin rates were about 30% lower than placebo rates. For these end-points, from 8 to 20 months, persantine aspirin rates were about 30% lower than aspirin rates, and aspirin patients entering within 6 months of the last myocardial infarction showed the larger percentage reduction in mortality.

From these recent studies, the general conclusion can be reached that nonfatal myocardial infarction is inhibited in myocardial infarction survivors receiving aspirin, or other drugs that seem to impair platelet aggregation, but the reduction in total mortality or even cardiac mortality—which is, in five of six studies, 20% or better—does not seem to achieve statistical significance. This may be because those patients who die late after myocardial infarction may include too many with myocardial insufficiency, irritable arrhythmogenic foci, and critical areas of myocardial ischemia whose course will not be significantly affected by a salutary influence on the platelet aggregation mechanism—even though that may inhibit progression of the disease (Jones, 1980).

Certain other questions have been raised by these studies that may also pertain to the basic issue: How early in convalescence after a myocardial infarction should inhibition of platelet aggregation begin? Is there an age or sex difference in any protection aspirin provides? By what mechanism is aspirin, dipyridamole, or sulfinpyrazone able to effect the course of coronary artery disease? Is there a preferred dose other than 0.9–1.0 g/day of aspirin (Jones, 1980)?

The MRC and AMIS studies included women as well as men. The former reported no significant difference in fatal or nonfatal cardiac events between aspirin and placebo groups in women aged 55–64 yr, whereas men in this age group showed the greatest improvement with aspirin (42%). In the AMIS study, no such sex difference was found, but the proportion of women was small (11%) and no difference was reported for either sex. In the MRC study, the enrolled women were older on the average than the men, and it is not known whether older patients may be less susceptible to aspirin prophylaxis while at greater risk of death. This issue needs further resolution.

Since aspirin in the doses that were utilized in many of these trials will both inhibit the formation of prostaglandin endoperoxides and thromboxane A_2, and the synthesis of prostacyclin, which is an inhibitor of platelet aggregation and a vasodilator that is found in the blood vessel wall, it has been suggested that a lower dose than 1.0 g/day of

aspirin might better achieve the first effect without the counterproductive latter effect. The minimum dose to achieve the desired inhibition of platelet aggregation should certainly be sought, for it would also minimize the undesirable gastrointestinal symptoms seen in about one-fifth of aspirin-treated patients.

One must conclude that aspirin may have a beneficial effect on the course of coronary artery disease subsequent to a myocardial infarction. It is indeed unfortunate that, after six carefully constructed trials in which such patients were randomized into aspirin and placebo groups, there remains no conclusive proof that ultimate mortality, the one statistic that carries great weight with statisticians and policymakers alike, is actually improved. The situation is strongly reminiscent of the long series of trials with anticoagulant therapy that has been similarly inconclusive. There may be good reason for this, because both treatments attempt to contravene the still incompletely understood thromboembolic contribution to the progression of atherosclerosis and the ultimate death of patients with coronary artery disease. Unfortunately further trials with aspirin are necessary to further explore its exact role in patients with coronary artery disease. However the Food and Drug Administration has recently accepted those controlled trials that establish the safety and efficacy of aspirin for control of transient ischemic attacks in the cerebral circulation and reducing the risk of their progression to stroke (Barnett, 1978; Fields *et al.*, 1977). With better trials one might expect similar protective effects on the coronary circulation to be seen with aspirin.

2. SULFINPYRAZONE. Sulfinpyrazone, an antigout drug that was developed during research on butazolidin, has been known to have potent platelet-inhibiting effects for some years (Smythe *et al.*, 1965). It has been shown to be useful in maintaining patency of arteriovenous shunts (Kaegi *et al.*, 1974) used for hemodialysis and to have a beneficial effect in patients with transient cerebral ischemic attacks (Evans, 1972). In 1972, Blakely and Gent (1975) presented the results of a prospective double-blind trial of sulfinpyrazone, 600 mg/day, in 291 elderly institutionalized men. They followed up the patients for 4 yr and found a statistically significant increase in survival among the patients with a previous history of atherosclerosis who had been treated with sulfinpyrazone in comparison with patients treated with placebo, especially among those who died of vascular causes. The numbers were too small to separate those who died of myocardial infarction for separate analysis.

In 1975, a large multicenter therapeutic trial of sulfinpyrazone was organized to determine if the drug had any value in preventing death during the first 1 or 2 yr after myocardial infarction. Data were accumulated in 1558 patients who were randomized to treatment with either placebo or sulfinpyrazone, 200 mg four times daily, starting 24–35 days after documented acute myocardial infarction. By the time recruiting was completed in July of 1977, it was apparent that there was a statistically significant benefit with sulfinpyrazone and the results of the study were reported. Although the data were examined at a mean of 8.4 months following entry into the trial (Anturane Reinfarcation Trial Research Group, 1978),the study was continued to its predetermined end—the study of all patients receiving therapy for a minimum of 1 and a maximum of 2 yr to assess both the duration of benefit and any hazards of long-term therapy (Anturane Reinfarction Trial Research Group, 1980). The patients were followed for an average of 16 months following their documented myocardial infarction.

All but one of the 106 deaths in the treatment groups were cardiac; 59 were sudden. The reduction in cardiac mortality compared to placebo at 24 months in the sulfinpyrazone group was 32% and the reduction in sudden death was 43%.

The benefits of sulfinpyrazone were attributable entirely to a reduction in sudden death during the second month to at least seven months after infarction, when there were 35 cardiac deaths in the placebo group and 17 in the sulfinpyrazone group; of these deaths, 24 in the placebo group and 6 in the sulfinpyrazone group were sudden cardiac deaths—a sulfinpyrazone-induced 74% reduction in the calculated mortality rate.

It was concluded by the participants in this trial that sulfinpyrazone prevented sudden cardiac death during the high-risk period shortly after an acute myocardial infarction, but there was no further apparent effect beyond the seventh month after infarction.

A number of criticisms were generated by this report. First, more patients in the placebo than in the treated group appeared to have had arrhythmias during infarction, and, although the differences were not statistically significant, there tended to be more patients with previous myocardial infarction, bundle branch block, angina pectoris, diabetes, and claudication among the placebo group. This has raised the question whether, by multivariate analysis, the placebo group might not prove to be significantly sicker than the treated group, and the results therefore spurious.

It is of interest to compare the impressive results of the sulfinpyrazone study with the outcome of the two cooperative trials using

aspirin (AMIS, PARIS) in postinfarction patients. Sulfinpyrazone and aspirin are similar in that both inhibit platelet prostaglandin synthesis, presumably because they inhibit platelet cyclooxygenase (although the mechanism involved in this inhibition may differ), and both are potent inhibitors of the second phase of platelet aggregation and the subsequent release reaction. Of course, in interpreting any discrepancy in the results of the trials, one should take into account differences between the pharmacologic actions of sulfinpyrazone and of aspirin as well as variations in experimental design. In the usual therapeutic doses, sulfinpyrazone does not affect bleeding time and tends to normalize the shortened platelet survival, whereas aspirin results in dose-dependent prolongation of bleeding time but does not affect platelet survival. In addition, aspirin is a strong irritant of the gastric mucosa. It has been noted that in the AMIS trial patients were admitted and treatment was begun as late as 8 months after infarction; in patients treated with sulfinpyrazone, on the other hand, the drug exerted the major effects in preventing sudden death within seven months. In the study reported by Elwood and Sweetnam (1979), which was more compatible to the sulfinpyrazone study with respect to the time of onset of treatment, the results were favorable in that the aspirin-treated patients had a significant reduction in total mortality and readmission to the hospital with nonfatal myocardial infarct. The PARIS study and the MRC study both found that mortality differences were greatest in the first 6 months after infarction and were nonevident subsequently. It is now well established that such patients are at greatest risk for death in the early months, so that the relatively low mortality as well as the absence of a difference in mortality in the AMIS study may be because some 85% of its patients were not enrolled until more than 6 months after the myocardial infarction (mean time 25 months). That an increase in mortality may yet be seen later in convalescence is supported by the earlier Coronary Drug Project study (1976), where most patients entered the trial some years after their latest infarction.

Sulfinpyrazone, which inhibits platelet aggregation but has no demonstrable effects on the electrophysiologic characteristics of normal cardiac cells, might have expected to reduce mortality from recurrent myocardial infarction rather than from sudden death, because sudden death is presumed to be due to cardiac arrhythmias. It is possible that these reversible episodes of platelet aggregation occur on ulcerated plaque, strategically narrowing a coronary artery and transiently reducing cardiac blood flow so that ischemia and a fatal arrhythmia can result. To support this story, Folts *et al.* (1976) has shown that drugs that inhibit the aggregation of platelets can prevent their accumulation

at sites where vessels are partly occluded. Could sulfinpyrazone's beneficial action in the postinfarction patient be coincidental and totally unrelated to its inhibitory effect on platelets, the characteristic that initially led to its application in this trial? Could it possibly prevent ischemia-induced arrhythmias without a demonstrable effect on nonischemic myocardium? In this connection, the preliminary experiences of Moschos *et al.* (1979) deserve attention. Using an experimental canine preparation in which a coronary artery was occluded but in which platelets did not accumulate in microcirculation, these investigators reported that pretreatment with sulfinpyrazone reduced the extent of myocardial ischemic injury, the extent of change in intracellular water, sodium, potassium concentrations during ischemia, and the frequency of death from arrhythmias. In a similar experimental study in which aspirin was employed instead of sulfinpyrazone, Moschos *et al.* (1978) showed that the frequency of deaths from arrhythmia was also decreased, but there was no apparent reduction in the extent of ischemic injury. In still another investigation, sulfinpyrazone was found to improve collateral blood flow in ischemic myocardium after experimental coronary occlusion in the dog (Davenport *et al.*, 1979). Clearly, further research is needed to elucidate the mechanism or mechanisms by which sulfinpyrazone and aspirin can exert their effects after infarction. A study similar to the American–Canadian sulfinpyrazone trial is currently underway in Italy; confirmatory data will be of great value.

3. DIPYRIDAMOLE. This is another drug with antiplatelet activity. (Emmons *et al.*, 1975) that has elicited interest among cardiologists. Although studies suggesting its efficacy in the prevention of emboli associated with prosthetic valves have been reported (Sullivan *et al.*, 1971), its value in the management of coronary artery disease has not yet been documented. In the trial reported above (PARIS), patients with documented infarction were randomized to treatment with either aspirin, dipyridamole and aspirin, or placebo. A persantine and aspirin combination seemed to reduce coronary artery mortality and the incidence of nonfatal myocardial infarction.

4. β-ADRENERGIC BLOCKING DRUGS. Some of these agents have also been demonstrated to have a significant platelet-inhibiting effect. The effects of propranolol on platelets have been well worked out (Frishman *et al.*, 1974; Weksler *et al.*, 1977). It appears not to be related to a β-adrenergic blocking effect but due to a nonspecific membrane activity shown in many β-adrenergic blocking drugs. This has been well docu-

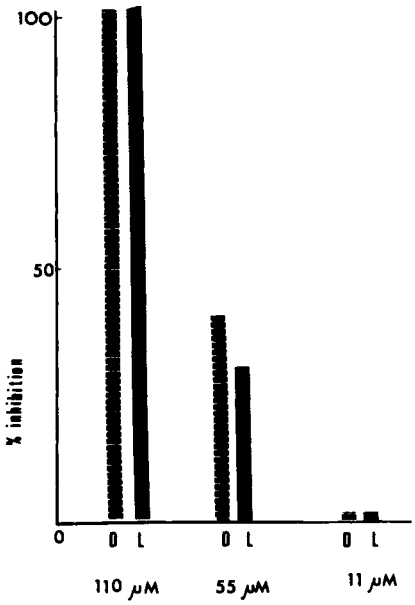

Fig. 2. Effects of D(+) and L(+) isomers of propranolol on platelet aggregability. The two isomers, in similar concentrations, were equipotent as inhibitors of platelet aggregation by ADP, and their effects did not significantly differ from the effect of racemic propranolol.

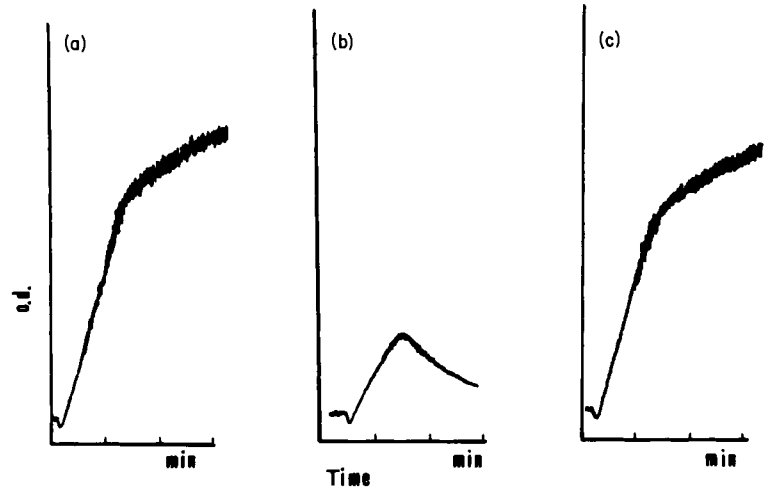

Fig. 3. Comparative effects of (a) saline, (b) propranolol, and (c) practolol on ADP-induced platelet aggregation. Practolol, which lacks membrane-stabilizing properties and has low lipid solubility, does not inhibit platelet aggregation even at 10 times the highest concentration of propranolol. The amount of platelet aggregation is proportional to the o.d. (optical density).

mented since D-propranolol, the part of racemic propranolol that has only membrane effects, is as potent a platelet inhibitor as the racemic mixture (D and L) itself (Fig. 2). Practolol, a drug with little membrane activity, has been shown not to effect platelets (Fig. 3) to any demonstrable degree (Frishman and Weksler, 1980).

B. Conclusion

One of the most exciting advances in the prophylaxis of acute myocardial infarction has been the introduction of platelet-inhibiting agents for treatment of patients following acute myocardial infarction. Although no conclusive data have been yet introduced, there is suggestive evidence that certain antiplatelet agents might be useful in preventing sudden death within the 6 months of an acute myocardial infarction. Whether or not these agents should be utilized in (1) patients with critical lesions on coronary arteries who are not candidates for bypass surgery, (2) patients who have had the intermediate coronary syndrome who do not undergo coronary angiography, (3) patients who have angina after coronary bypass surgery, (4) all patients with new angina, (5) all patients within 30 days of coronary bypass surgery, and (6) all patients with chronic angina has yet to be established in clinical trials.

VII. CORONARY ARTERY SURGERY

The advances in the surgical treatment of coronary artery disease constitutes an important development in the 1970s. Mortality from elective coronary vascularization has declined to about 1% in most centers and it is clear that patient survival can be prolonged when coronary artery bypass is performed by a skilled surgical team in certain subsets of patients, notably those with obstructive lesions to the left main coronary artery (Takaro *et al.*, 1976), and perhaps those with obstructions of all three major coronary arteries (European Coronary Surgery Study Group, 1979). Whether these considerations apply specifically to postinfarction patients, especially those with no or minimal angina pectoris, remains to be established. Fortunately, the coronary artery surgery being conducted by the National Heart, Lung, and Blood Institute is addressing this critically important question.

VIII. SUMMARY

In the 1980s, we can now offer patients far greater diagnostic and therapeutic possibilities for the evaluation and prevention of myocardial infarction and sudden death. Great progress has been made toward prolonging the life of postinfarction patients but it is very unlikely that a single treatment will have been found to meet the needs of these patients. Coronary artery disease is far too complex and myocardial infarction can be caused by multiple mechanisms (thrombosis, embolism, spasm, etc.). The mechanisms of arrhythmogenesis also appears to be quite complex. A single effective therapeutic measure would have to be capable of preventing all these conditions.

In the future, patients will have to be classified into specific treatment subgroups: (1) patients vulnerable to serious arrhythmias and sudden death, (2) those most likely to have repeated coronary occlusions, (3) those at high risk of dying from pump failure, (4) those in whom ischemia and infarction are spasm mediated, (5) those in whom a disorder of the clotting mechanism may be operative in ischemia and infarction, and (6) those at low risk of infarction and sudden death. Once the patient has been classified, specific treatments can be tailored to individual requirements.

Newer pharmacological modalities are on the horizon: the calcium entry blockers (verapamil, nifedipine, lidoflazine), which block the slow calcium current on myocardial cells and coronary vascular smooth muscle, as well as a number of recently developed antiarrhythmic agents. Better trials also must be carried out with the modalities currently available to us. The medical and surgical treatment for prevention of sudden death and myocardial infarction is ever ripe for new and imaginative approaches.

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4 Pharmacology of Antiplatelet Agents and Their Role in Coronary Disease

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I. SUMMARY OF CLINICAL TRIALS OF ANTIPLATELET AGENTS

Several large clinical trials have been completed in which drugs known to affect platelet function were tested for their influence on the

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frequency of subsequent coronary events in patients who had had a myocardial infarction (Elwood *et al.*, 1974; Coronary Drug Project Research Group, 1976; Breddin *et al.*, 1979; Elwood and Sweetnam, 1979; Aspirin Myocardial Infarction Study Research Group, 1980; Persantine-Aspirin Reinfarction Study, 1980; Anturane Reinfarction Trial, 1978, 1980). Aspirin, in doses ranging from 300 mg to 1.5 g/day, was used in most of the trials, aspirin plus dipyridamole was compared with aspirin or placebo in the Persantine-Aspirin Reinfarction Study (1980), and sulfinpyrazone was used in the Anturane Reinfarction Trial (1978, 1980) and the Anturan Reinfarction Italian Study (1982). There have also been clinical trials of drugs such as clofibrate (Committee of Principal Investigators, 1978; Oliver *et al.*, 1980) and coumarin anticoagulants (Loeliger *et al.*, 1967; Meuwissen *et al.*, 1969; Gross *et al.*, 1972; Peto, 1978; Sixty Plus Reinfarction Study Research Group, 1980); these drugs inhibit platelet reactions less directly and the original rationale for their use did not include their effects on platelets.

The trials of aspirin, aspirin plus dipyridamole, and sulfinpyrazone were initiated in the early 1970s and were based on several assumptions that were reasonable at that time, in the light of the available knowledge. These assumptions were (1) that clinical complications of atherosclerosis are primarily caused by thromboembolic events, (2) that exposure of collagen in the vessel wall to blood is a key factor in the initiation and growth of thrombi, and (3) that the frequency of occurrence of thromboembolic events in arteries could be reduced with drugs that inhibit platelet aggregation in response to agents such as collagen.

The results of these clinical trials are summarized in Table I and it is apparent that they are far from conclusive. The largest aspirin trial, the Aspirin Myocardial Infarction Trial (AMIS) (1980) sponsored by the National Institutes of Health in the United States, showed a slightly increased percentage of deaths from all causes in patients given aspirin. The published conclusion was that "aspirin is not recommended for routine use in patients who have survived a myocardial infarction."

Recently, however, Peto (1978) has analyzed all six trials of aspirin in postmyocardial infarction patients by comparing the number of deaths observed among the aspirin-takers with the number that would have been expected if the probability of death were unaffected by aspirin (Editorial, 1980). He concluded that aspirin administration does reduce the risk of cardiovascular death at the level of significance of $p < 0.01$ and reduces reinfarction even more significantly ($p < 0.0001$). The benefit of aspirin is similar to that achievable with anticoagulants—prevention of about one-sixth of the deaths (Peto, 1978).

TABLE I

Results of Clinical Trials of Aspirin, Aspirin Plus Dipyridamole, or Sulfinpyrazone in Post-Myocardial Infarction Patients

Trial	Dose of drug (mg)	Entry time after myocardial infarction	Time observed (yr)	Number of patients in trial	Nonfatal myocardial infarction (% of patients)		Death (% of patients)					
							All causes		Myocardial infarction		Sudden	
					Drug	Placebo	Drug	Placebo	Drug	Placebo	Drug	Placebo
MRC I Elwood et *al.* (1974)	ASA 300 daily	1–6 months	2.5	1239	—	—	8.3	10.9	—	—	—	—
MRC II Elwood and Sweetnam (1979)	ASA 300 t.i.d.	50% within 7 days	1.0	1682	7.1	10.9	12.3	14.8	—	—	—	—
Coronary Drug Project Aspirin Study (1976)	ASA 324 t.i.d.	75% ≥ 5 yr	0.83–2.3	1529	3.7	4.2	5.8	8.3	—	—	2.6	3.2
German–Austrian (1979)	ASA 1500 daily	30–42 days	2	620	3.5	4.9	8.5	10.6	1.6	3.2	2.5	3.9
AMIS (1980)	ASA 500 b.i.d.	2–60 months	3	4524	6.3	8.1	10.8	9.7	6.0	6.0	2.7	2.0
PARIS (1980)	ASA 324 t.i.d.	2–60 months	3–4	1216	6.9	9.9	10.5	12.8	2.5	5.7	5.6	4.4
PARIS (1980)	ASA 324 t.i.d. + dipyridamole 75 t.i.d.	2–60 months	3–4	1216	7.9	9.9	10.7	12.8	4.0	5.7	3.7	4.4
ART (1978, 1980)[a]	Sulfinpyrazone 200 q.i.d.	25–35 days	1–2	1558	—	—	5.7	7.9	2.2	2.3	2.8	4.7
ART (1978, 1980)[b]	Sulfinpyrazone 200 q.i.d.	25–35 days	1–2	1629	—	—	9.1	10.9	—	—	—	—
ARIS (1982)[b]	Sulfinpyrazone 400 b.i.d.	15–25 days	1–2	727	3.2	7.7	5.4	5.8	0.8	1.6	3.0	2.5

[a] Values are for analyzable deaths of eligible patients. Sudden death is recorded for the total time of the study.

[b] Values are for all randomized patients whether eligible or not, and for all deaths whether analyzable or not.

In the first sulfinpyrazone trial, the drug did not have a statistically significant effect on overall mortality but it did significantly reduce the incidence of sudden death in the first six months after a myocardial infarction (Anturane Reinfarction Trial, 1980). The more recent Italian Study, however, showed somewhat different results with sulfinpyrazone in postmyocardial infarction patients (Anturan Reinfarction Italian Study, 1982); no effect on sudden death was apparent, but reinfarction was significantly less in the sulfinpyrazone-treated group in comparison to the placebo-treated group. Total mortality, however, was not significantly different in the two groups.

The designs and analyses of all the trials differed from each other and thus direct comparisons cannot be made. It is evident, however, that any beneficial effects that the drugs may have had were not dramatic in the patient populations that were enroled in these trials. Retrospective analysis, however, indicates that some subgroups of patients may have derived more benefit from the drugs than the whole group. Thus the tantalizing possibility remains that the clinical complications of coronary disease in some patients may be thromboembolic in nature and susceptible to inhibition by these drugs that affect platelet reactions.

Evidence that these drugs may be effective when thromboembolism is the cause of clinical complications also comes from the results of the clinical trials of aspirin in patients at risk of stroke, and of aspirin or sulfinpyrazone in dialysis patients at risk of thrombosis of arteriovenous shunts. In the two studies in which aspirin was administered to patients considered to be at risk of stroke because they were experiencing transient attacks of cerebral ischemia, the incidence of subsequent transient ischemic attacks, stroke, and death was significantly reduced, particularly in the male patients (Fields *et al.*, 1977; Canadian Cooperative Study Group, 1978). Thromboembolism is recognized as responsible for at least some of these transient ischemic attacks (Barnett, 1976), so it seems likely that aspirin reduced the incidence or extent of thrombosis in these patients. In contrast, sulfinpyrazone was not found to be effective (Canadian Cooperative Study Group, 1978). Both drugs, however, reduced the number of thrombi in arteriovenous shunts of dialysis patients, indicating that they both have inhibitory effects on thrombosis of the type that occurs under these circumstances (Kaegi *et al.*, 1975; Harter *et al.*, 1979).

It is not possible to incorporate all of the observations from all of these clinical trials into a simple theory that thromboembolism is solely responsible for the clinical complications of arterial disease in the coronary and extracranial vessels. Other mechanisms must also

operate and these mechanisms may not directly involve platelets nor be affected by drugs that inhibit platelet reactions. One of these mechanisms may be coronary artery spasm (Braunwald, 1978; Maseri *et al.*, 1978, 1980; Antman *et al.*, 1980).

The following questions arise from the results of the clinical trials: (1) Is thromboembolism responsible for some, but not all, of the clinical complications? (2) Can subgroups of patients be identified in which thromboembolism is likely to be the primary event? (3) Do the mechanisms in the initiation and growth of thrombi vary sufficiently that different types of thromboembolism should be recognized? (4) Are tbere some types of thrombosis in which platelets play a major role, and other types in which coagulation is the most important factor? (5) Will drugs that inhibit platelet reactions reduce the incidence of thromboembolism and be effective in all individuals? (6) If there are several causes of thrombosis, which platelet reactions are most likely to be dominant in each, and hence which drugs are most likely to be effective? In considering this sixth question, it is necessary to review our current concepts of the platelet reactions involved in the formation of thrombi in arteries.

II. THROMBUS FORMATION IN ARTERIES

Thrombosis involves the response of blood to vessel wall injury (Mustard and Packham, 1979). Thrombi do not form on normal, undamaged endothelium. Two aspects of thrombus formation will be considered—thrombus formation at an injury site on a normal vessel, and thrombus formation on a repeatedly damaged vessel wall. Studies with experimental animals have involved a great many different ways of causing vessel wall injury but, in nearly all cases, otherwise normal vessels of young animals have been used. In only a few studies has thrombus formation on the thickened intima of repeatedly damaged vessels been examined. It is now apparent that the reactions of the blood with such a surface are quite different than with a normal vessel subjected to a single injury. It seems likely that the reactions of the damaged thickened intima will be similar to those of an atherosclerotic vessel wall.

When the endothelium of a normal artery is damaged or removed, platelets adhere to the subendothelial constituents—collagen, microfibrils, and basement membrane (Baumgartner *et al.*, 1976; Groves *et*

al., 1979). If blood flow is undisturbed, only a single layer of platelets covers the subendothelium, but if flow is disturbed platelet aggregates, with or without associated fibrin, may form. Current theory holds that platelets that adhere to exposed collagen in the subendothelium release some of their granule contents (Hovig, 1963) and form the short-lived aggregating and release-inducing agent thromboxane A_2 (Smith *et al.*, 1974a; Hamberg *et al.*, 1975). Among the released granule contents are ADP and serotonin; ADP causes platelet aggregation and, although serotonin is a weak aggregating agent by itself, it potentiates the effects of other aggregating agents (O'Brien, 1964; Baumgartner and Born, 1968). ADP, thromboxane A_2, and serotonin accumulate in vortices in regions of disturbed flow and cause platelets in the blood at these sites to change shape and adhere to each other and to the platelets that are already adherent to the subendothelium. Around the platelet aggregate attached to the wall, the coagulation pathway is activated in a number of ways and thrombin forms locally. The extrinsic coagulation pathway is activated by tissue thromboplastin (Nemerson and Pitlick, 1972), while factor XII of the intrinsic pathway is activated by exposed collagen (Niewiarowski *et al.*, 1966), and by the aggregated platelets (Walsh, 1972a; Walsh and Griffin, 1981): factor XI may also be activated on platelets adherent to collagen (Walsh, 1972b; Walsh and Griffin, 1981); platelet factor 3 (a phospholipoprotein) becomes available on the surface of platelets that have undergone a release reaction (Joist *et al.*, 1974) and accelerates two steps of the intrinsic coagulation pathway (Zwaal, 1978; Jackson and Nemerson, 1980). Thrombin has several effects (Packham *et al.*, 1978). It causes further release of platelet granule contents and the synthesis of more thromboxane A_2 by the platelets. Thrombin itself can also cause platelets to aggregate through a mechanism that is independent of released ADP or thromboxane A_2 (Packham *et al.*, 1977; Kinlough-Rathbone *et al.*, 1977a). Thus, thrombin causes further platelet aggregation and release of granule contents. In addition, thrombin converts fibrinogen to fibrin. Polymerizing fibrin adheres to the platelets (Niewiarowski *et al.*, 1972) and fibrin stabilizes the platelet aggregate and prevents it from being broken up by the force of the flowing blood. Platelet aggregates that are not stabilized by fibrin readily deaggregate. This theory of arterial thrombus formation in normal vessels has been developed from many *in vivo* and *in vitro* experiments. Although exposed collagen has an initiating role in this theory, thrombin has a major role in the growth and stabilization of the thrombi.

Electron microscopy of thrombi that form on repeatedly injured vessels shows a somewhat different picture (Stemerman, 1973; Groves *et al.*, 1982b). Fibrin is usually apparent between the platelet aggregates

and the injured wall as well as being interspersed among and around the aggregates. It therefore seems likely that thrombin generation and fibrin formation may be mainly responsible for the initiation as well as the growth of these thrombi. If this type of thrombus formation also occurs in diseased arteries, it is unlikely that drugs that act mainly by inhibiting thromboxane A_2 formation would have much effect. Aspirin and sulfinpyrazone inhibit platelet aggregation that is largely dependent on thromboxane A_2 formation, but they have little or no effect on thrombin-induced platelet aggregation (Evans *et al.*, 1968; Holmsen *et al.*, 1975) or on the formation of fibrin. These drugs, therefore, are unlikely to inhibit thrombosis that is mainly the result of thrombin formation. These considerations were unknown when the clinical trials of these drugs were initiated, but the results of the trials are in keeping with the suggestion that these drugs may inhibit only some types of thrombus formation.

ADP and epinephrine are two other agents that induce platelet aggregation, but the primary phase of aggregation induced by these agents is not affected by drugs such as aspirin and sulfinpyrazone that prevent thromboxane A_2 formation. ADP is released from platelets but is also lost from damaged cells and hemolyzing red blood cells at injury sites. Hemorrhage into ruptured atherosclerotic plaques is accompanied by hemolysis and local ADP-induced aggregation (Constantinides, 1966; Crawford, 1977). Recent studies by Born and Wehmeier (1979) have provided experimental evidence that hemolysis may have a role in the initiation of some types of thrombi. Epinephrine acts synergistically with other aggregating agents (Ardlie *et al.*, 1966; Haft *et al.*, 1972). It also affects vessel wall tone and myocardial metabolism (Maling and Highman, 1958; Nahas *et al.*, 1958) and accelerates blood coagulation (Özge *et al.*, 1966). Thus the effects of stress and the resulting epinephrine increase may contribute to platelet aggregation. Drugs that inhibit the action of epinephrine may exert some of their effect by diminishing platelet responses.

III. THROMBOEMBOLISM AND CLINICAL COMPLICATIONS OF CORONARY DISEASE

Thrombi that cause clinical complications may be occlusive or nonocclusive; the latter may embolize into the microcirculation. Occlusive thrombi in severely diseased coronary arteries are believed by many

investigators to cause myocardial infarction (Jørgensen *et al.*, 1968; Friedman, 1970; Chandler *et al.*, 1974; Fulton and Sumner, 1977), although contrary evidence has been published (Silver *et al.*, 1980). For their formation, it seems likely that strong stimulation of platelet aggregation and thrombin and fibrin formation would have to occur. It has been suggested that vasospasm may initiate thrombosis and subsequent myocardial infarction (Maseri *et al.*, 1978).

Emboli from nonocclusive thrombi have been suggested as one of the causes of sudden cardiac death (Haerem, 1978). Occlusive thrombi or gross thrombosis of coronary arteries are rarely observed in association with sudden death. Many other possible causes have been considered on the basis of experimental evidence, including vasospasm that may or may not be associated with vessel wall damage and formation of the vasoconstrictor, thromboxane A_2, by platelets, or leukocytes.

Studies with animals have shown that platelet emboli or emboli from mural thrombi can obstruct the microcirculation of the heart and cause ventricular fibrillation, sudden death, or myocardial lesions similar to those observed in humans who die suddenly (Jørgensen *et al.*, 1967a; Moore *et al.*, 1976a; Moschos *et al.*, 1978). It is not known how often these events are responsible for sudden death in patients with coronary artery disease.

IV. EFFECTS OF SUBSTANCES PRODUCED BY INJURED VESSEL WALLS

Injured vessel walls both promote and limit thrombosis. As already discussed, thrombosis is promoted by platelet adherence and thrombin generation at an injury site. However, injury of endothelial and smooth muscle cells stimulates them to produce prostacyclin (PGI_2), a potent inhibitor of platelet aggregation and the release reaction and a vasodilator (Moncada and Vane, 1978). Thrombin also stimulates these cells to produce PGI_2 (Weksler *et al.*, 1978). PGI_2 not only inhibits platelet aggregation in response to ADP, collagen, thrombin, and all other known aggregating agents, but also inhibits the release of platelet granule contents (Moncada *et al.*, 1976; Moncada and Vane, 1978; Marcus, 1979). In addition, somewhat higher concentrations of PGI_2 have been shown to inhibit platelet adherence to the subendothelium (Higgs *et al.*, 1978; Cazenave *et al.*, 1979; Weiss and Turitto, 1979) and to prevent the initial change in platelet shape that occurs in response to aggregat-

ing agents (Ehrman and Jaffe, 1980). Inhibition of shape change by PGI_2 is thought to limit the availability of the membrane sites on the platelets that are normally responsible for acceleration of coagulation reactions when platelets have been stimulated (Ehrman and Jaffe, 1980; Bunting and Moncada, 1980). Thus PGI_2 production may limit the extent of thrombosis at an injury site; evidence for this has been obtained in experimental animals under conditions of stasis in severely injured veins (Kelton *et al.*, 1978a). PGI_2 may also inhibit thrombosis in the microcirculation (Rosenblum and El-Sabban, 1978; Bourgain, 1978, 1979, 1980). It is unlikely that PGI_2 has a major effect in limiting thrombosis in high-pressure, high-flow conditions in arteries where PGI_2 is removed quickly by the flowing blood. Aspirin and other nonsteroidal antiinflammatory drugs block PGI_2 formation but, since PGI_2 was unknown when the clinical trials were initiated, possible effects of the drugs on PGI_2 formation can only be considered retrospectively in relation to the trials.

Plasminogen activator is another substance produced by injured vessel walls that affects thrombi (Majno and Joris, 1978; Laug, 1979). Under its influence, plasmin forms in and around the thrombus, lyses the fibrin holding the thrombus together, and thus promotes embolization under the force of blood flow. Although this will reduce the size of the thrombus, the emboli may cause ischemia when they impact in the downstream microcirculation. Both sulfinpyrazone and aspirin have been shown experimentally to promote fibrinolysis (Rüegg, 1976; Moroz, 1977).

V. THE ROLE OF PLATELETS IN THE INITIATION OF CORONARY ARTERY DISEASE

Although the large platelet component of arterial thrombi has resulted in intense interest in the contributions of platelet aggregation and thromboembolism to the clinical complications of atherosclerosis, it should be kept in mind that platelets are also now recognized as having a role in the initiation and progression of atherosclerotic lesions. The demonstration by Ross and his colleagues (1974) that platelets can release a factor that causes smooth muscle cells and fibroblasts to proliferate has reinforced the earlier concept that platelet thrombi on vessel walls contribute to the development of atherosclerotic lesions

(Mustard *et al.*, 1964; Mustard and Packham, 1975). Moore and coworkers (1976b) have added further support to this theory by their observations that thrombocytopenia, induced in normolipemic rabbits, prevents the development of lipid-rich atherosclerotic lesions in aortae subjected to repeated injury by an indwelling catheter.

A few studies have been reported of the effects of various drugs on smooth muscle cell proliferation in injured blood vessels of experimental animals but the results are not entirely consistent. Sulfinpyrazone (but not aspirin or dipyridamole) was found by Baumgartner and Studer (1977) to reduce smooth muscle cell proliferation in de-endothelialized rabbit arteries; heparin (but not aspirin) had a similar effect in rats (Clowes and Karnovsky, 1977a,b; Guyton *et al.*, 1980). Clopath (1980) reported recently that aspirin inhibited intimal proliferation in repeatedly injured aortae of miniature swine. Harker (1980) has reported that dipyridamole or sulfinpyrazone limits the extent of intimal proliferation in baboons given continuous infusions of homocysteine and that sulfinpyrazone has this effect in hypercholesterolemic monkeys. In dogs and monkeys, the combination of aspirin and dipyridamole has been found to reduce intimal thickening in coronary bypass vein grafts (Metke *et al.*, 1979; McCann *et al.*, 1980). Pick and her colleagues (1979) observed that aspirin inhibits the development of coronary atherosclerosis (but not aortic atherosclerosis) in cynomolgus monkeys fed an atherogenic diet. These drugs inhibit platelet reactions either directly or by blocking the actions of thrombin, but it is not established that they exert their effects only through platelets. Obviously such studies cannot be done in man. The clinical trials provide no information concerning the effects of drugs on the development of atherosclerotic lesions because all the trials have involved patients who have already experienced clinical complications and therefore presumably have long-established atherosclerotic lesions. It is not reasonable to suggest long-term drug administration to prevent the development of lesions, since early lesions have been observed in children (Burch, 1978), and it is not possible to identify the population at risk, except perhaps by family history, smoking habits, and serum cholesterol levels. Lessening vessel wall injury by modification of life style factors such as smoking and diet would seem to be a more feasible approach to inhibiting the development of atherosclerotic lesions (Stamler, 1979; Hjermann *et al.*, 1981).

It should also be mentioned that the resolution and incorporation of persistent thrombi into vessel walls can contribute to thickening of the walls, narrowing of the lumen, and stenosis (Jørgensen *et al.*, 1967b; Mustard and Packham, 1979). This is probably important in the devel-

opment of advanced atherosclerotic lesions. In the presence of drugs that inhibit platelet reactions, thrombi that do form may be smaller and thus contribute less to vessel wall thickening.

VI. TESTS OF THE EFFECTS OF DRUGS ON PLATELET REACTIONS

Before considering the specific biochemical reactions in platelets that some drugs are known to affect, it is useful to review some of the many tests of platelet function *in vitro* and *in vivo* that have been employed. Historically, *in vitro* tests became relatively easy to do when devices were developed to measure and record the increase in light transmission through stirred platelet-rich plasma that occurs as platelets aggregate in response to ADP, epinephrine, collagen suspensions, thrombin, and other less physiologic aggregating agents. Effects of drugs on aggregation can be readily tested by adding them to the platelet suspension before the aggregating agent. This is the most frequently used test but, when it is used with human citrated platelet-rich plasma, it overemphasizes the importance of thromboxane A_2 in platelet aggregation. The reason for this is that, when human platelets are brought into close contact in a medium with a low concentration of ionized calcium, they are stimulated to produce thromboxane A_2 which causes platelet aggregation and the release reaction and potentiates the effects of other aggregating agents (Mustard *et al.*, 1975; Macfarlane *et al.*, 1975; Kinlough-Rathbone *et al.*, 1977b). Human platelets in citrated platelet-rich plasma exhibit two phases of aggregation in response to ADP or epinephrine—a primary phase that is independent of thromboxane A_2 and is unaffected by drugs that inhibit its formation, and a secondary phase that involves thromboxane A_2 and the release of some of the platelet granule contents. In media containing a physiologic concentration of ionized calcium, only the primary phase occurs in response to ADP and drugs that block thromboxane A_2 formation are not inhibitory. It seems likely that this is the situation *in vivo* in circumstances in which ADP makes a large contribution to platelet aggregation.

Collagen-induced aggregation in this *in vitro* test system is largely mediated by thromboxane A_2 formation and released ADP, both in the presence of a physiologic concentration of ionized calcium and in citrated platelet-rich plasma (Kinlough-Rathbone *et al.*, 1977a). It should

be kept in mind that the majority of the platelets do not become adherent to collagen under the conditions generally used in this test and that an "aggregometer" records the reactions of these platelets that are not adherent to collagen. Although drugs that inhibit thromboxane A_2 formation inhibit the release of platelet granule contents in this system, it has been shown that these drugs do not inhibit the release reaction of platelets that actually adhere to collagen (Kinlough-Rathbone *et al.*, 1980). This is an important consideration in extrapolating to an *in vivo* situation where platelets adhere to exposed collagen at an injury site. Drugs that inhibit thromboxane A_2 formation would not prevent the release from the adherent platelets of the factor that causes smooth muscle cell proliferation and is undoubtly involved in the development of atherosclerotic lesions at sites of repeated vessel wall injury.

Effects of drugs on the release of platelet granule contents can be assessed by measuring the extent of release of radioactive serotonin from prelabeled platelets, or by radioimmunoassay of the amounts of platelet factor 4 or β-thromboglobulin that are released (Bolton *et al.*, 1976; Levine and Krenz, 1977; Kaplan *et al.*, 1978; Rucinski *et al.*, 1979). Both of these proteins are contained in the α granules of platelets and are considered to be unique to platelets. Thromboxane A_2 formation is determined by assaying its more stable product, thromboxane B_2, by radioimmunoassay or gas chromatography (Ferraris *et al.*, 1977; Ali *et al.*, 1977).

Many drugs and other agents have been identified that inhibit platelet aggregation and the release reaction (Mustard and Packham, 1978). Few of these are suitable for long-term administration to man. The drugs that were used in the major clinical trials were not designed as inhibitors of platelet function and were in clinical use for other purposes long before their effects on platelets were recognized.

Other *in vitro* tests of platelet function have also been developed. One of the main types involves measurements of the number of platelets that adhere to various surfaces (glass beads, polymer surfaces designed to be nonthrombogenic, collagen, and the subendothelium). The contribution of activated platelets to blood coagulation has also been assessed.

These *in vitro* tests of platelet function can also be done with platelets isolated from the blood of experimental animals or man after drug administration. Additional information can be gained in this way concerning the effectiveness of concentrations that can be achieved *in vivo*, the length of time that effective concentrations of a drug or its active metabolites remain in the circulation, and side effects. It should

be emphasized, however, that drug metabolism differs from species to species and that the production of an active drug metabolite in an experimental animal does not always indicate that effective amounts of the metabolite will be produced in man.

A large number of methods to produce thrombosis in experimental animals have been developed to permit the testing of drugs *in vivo*. Results with different methods have often varied from inhibition, to no effect, to enhancement, depending on dosages, sites where thrombi are induced (arteries, veins, microcirculation), methods of injury, and the species. Some of these differences in experimental techniques may determine whether ADP, thrombin, collagen, or thromboxane A_2 have the major role in thrombus formation and whether PGI_2 limits thrombus formation. The mechanisms that are mainly responsible for thrombosis in large arteries where blood flow is rapid and laminar undoubtedly differ from those in veins and in the microcirculation. Thus it is not surprising that contradictory results have sometimes been reported for the same drug by different investigators.

Tests of platelet function *in vivo* in man after drug administration are necessarily limited. They include bleeding time, platelet survival and turnover, plasma concentrations of platelet factor 4, β-thromboglobulin and thromboxane B_2, and determination of the number of circulating platelet aggregates. The platelet hypersensitivity to aggregating agents that has frequently been reported in association with the clinical complications of atherosclerosis is almost always reduced by the administration of drugs that inhibit thromboxane A_2 formation (Packham, 1978). However, if platelet hypersensitivity is a result rather than the cause of the clinical complications, it should not be concluded that such drugs would reduce the incidence of clinical complications.

VII. DRUGS THAT INHIBIT PLATELET REACTIONS

Drugs that inhibit platelet reactions can be arbitrarily divided into four broad categories: (1) drugs that inhibit thromboxane A_2 formation; (2) drugs that raise the cyclic AMP concentration in platelets; (3) drugs that act through other mechanisms, some of them not yet defined; and (4) drugs that inhibit platelet function indirectly by preventing thrombin formation or its actions on platelets and fibrinogen.

A. Drugs That Inhibit Thromboxane A_2 Formation

The drugs that have been most extensively tested in large clinical trials—aspirin and sulfinpyrazone—are in this category. Other nonsteroidal antiinflammatory drugs such as phenylbutazone, indomethacin, ibuprofen, fenoprofen, and naproxen act in a similar fashion. These drugs inhibit cyclooxygenase, the enzyme that converts arachidonate to prostaglandin G_2, the precursor of thromboxane A_2. Figure 1 illustrates the reactions involved in the liberation of arachidonate from membrane phospholipids under the influence of activated phospholipases and its conversion in platelets to a number of products. Three types of enzymes involved in the production of thromboxane A_2 can be inhibited by drugs: phospholipases, cyclooxygenase, and thromboxane synthetase.

1. Inhibitors of Phospholipases and Diglyceride Lipase. The first step in thromboxane A_2 formation is the hydrolysis of arachidonate in membrane phospholipids. Until recently, the enzyme responsible was thought to be phospholipase A_2 (Bills *et al.*, 1977; Blackwell, 1978) but several investigators have now demonstrated that phospholipase C, followed by diglyceride lipase, may also catalyze the freeing of arachidonate (Rittenhouse-Simmons, 1979; Bell *et al.*, 1979; Lapetina and Cuatrecasas, 1979). The relative importance of the two phospholipases is not known. Their activation is thought to be dependent on the freeing of internal platelet calcium from sites of sequestration into the cytosol, under the influence of agents such as collagen and thrombin. Experiments with purified phospholipase A_2 from human platelets have demonstrated that indomethacin, sodium meclofenamate, and sodium flufenamate (but not aspirin) inhibit the activity of this enzyme (Jesse and Franson, 1979; Franson *et al.*, 1980).

When platelet phospholipids have been prelabeled by incubation with radioactive arachidonate, several drugs have been shown to inhibit the freeing of arachidonate in response to platelet stimulation. It is now unclear whether these drugs affect phospholipase A_2, phospholipase C, or both enzymes. Drugs that have been recognized to act in this way include indomethacin (but not aspirin) (Deykin and Russell, 1978), the antimalarial quinacrine hydrochloride (mepacrine hydrochloride) (Blackwell, 1978; Winocour *et al.*, 1979), and steroidal antiinflammatory drugs (Hong and Levine, 1976; Flower, 1978). Hydrocortisone 21-sodium succinate and methyl prednisolone sodium succinate

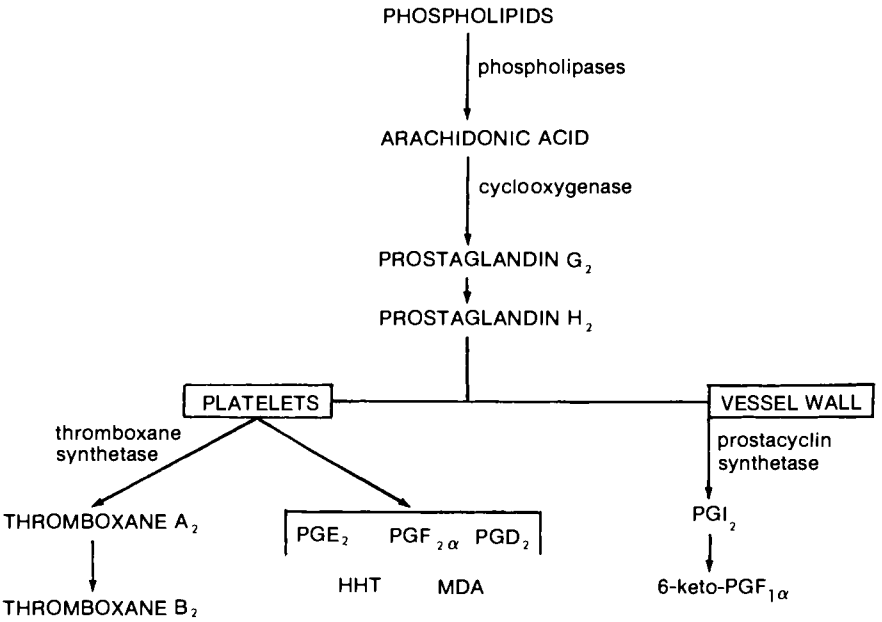

Fig. 1. Formation of thromboxanes and prostaglandins by platelets and by cells of the vessel wall: HHT is 12-L-hydroxy-5,8,10-heptadecatrienoic acid; MDA is malondialdehyde.

probably inhibit platelet aggregation by inhibiting phospholipase and thus preventing thromboxane A_2 formation (Pierce *et al.*, 1974; Nelson and Taylor, 1975; Hong and Levine, 1976; Flower, 1978; Jørgensen and Stoffersen, 1980). However, the steroidal antiinflammatory drugs undoubtedly have other effects on platelets in addition to inhibition of thromboxane A_2 formation, since in high concentrations they can inhibit platelet aggregation and release in response to all aggregating agents, including thrombin, and inhibit the adherence of platelets to collagen and the subendothelium (Cazenave *et al.*, 1976). Pretreatment of platelets with methyl prednisolone or hydrocortisone reduces the ability of the platelets to shorten the prolonged bleeding time of thrombocytopenic rabbits (Cazenave *et al.*, 1976). There are indirect indications in man and primates that corticosteroids may be beneficial in conditions to which platelet aggregates contribute. To our knowledge, there are no reports of the effects of these drugs in coronary artery disease. Indomethacin has also been shown to inhibit the diglyceride lipase in platelets that acts after phospholipase C (Rittenhouse-Simmons, 1980).

2. Inhibitors of Thromboxane Synthetase. The conversion of prostaglandin H_2 to thromboxane A_2 is catalyzed by thromboxane synthetase (Fig. 1) (Gorman, 1980). The inhibitors of this enzyme that have been identified include imidazole and its derivatives 9,11-azoprosta-5,13-dienoic acid, pinane thromboxane A_2, burimamide, and levamisole, an antihelmintic drug (Moncada *et al.*, 1977; Needleman *et al.*, 1977a; Tai and Yuan, 1978; Hokama *et al.*, 1978; Nicolaou *et al.*, 1979; Allan *et al.*, 1980; Vermylen *et al.* 1981; Tyler *et al.*, 1981). Imidazole and pinane thromboxane A_2 have been shown to protect ischemic myocardial tissue during acute myocardial ischemia in cats, probably through inhibition of thromboxane synthesis (Smith *et al.*, 1980; Schrör *et al.*, 1980).

3. Cyclooxygenase Inhibitors. Most of the nonsteroidal antiinflammatory drugs and the uricosuric agent sulfinpyrazone, which has weak antiinflammatory properties, inhibit cyclooxygenase (Fig. 1). Aspirin irreversibly acetylates the enzyme and its inhibitory effect persists during the life of the platelets that have been exposed to it (O'Brien, 1968; Burch and Majerus, 1979). Surprisingly, although sodium salicylate has antiinflammatory properties, it has little or no inhibitory effect on cyclooxygenase (Weiss *et al.*, 1968), although it must react with the enzyme because it bas been reported to protect the enzyme from acetylation by aspirin (Vargaftig, 1978; Merino *et al.*, 1980). Sulfinpyrazone, indomethacin, and diflunisal also interfere with acetylation of cyclooxygenase by aspirin (Stanford *et al.*, 1977; Majerus and Stanford, 1977; Ali and McDonald, 1979). Metabolites of sulfinpyrazone have been identified that inhibit cyclooxygenase and sufficient concentrations of these may be formed in man to contribute significantly to the inhibitory effects (Buchanan *et al.*, 1978; Pedersen and Jacobsen, 1979; Butler *et al.*, 1980). Indomethacin is the most potent inhibitor of cyclooxygenase but it appears to have additional effects on platelet reactions, such as inhibition of phospholipase A_2 (see above). Its side effects preclude chronic administration to large numbers of patients.

Aspirin and sulfinpyrazone have received more attention as inhibitors of platelet function than any of the other drugs that affect platelets. They were among the first drugs recognized to inhibit the interaction of platelets with collagen, although at the time nothing was known of the arachidonate pathway or thromboxane A_2 production (Packham *et al.*, 1967; Evans *et al.*, 1967, 1968; Weiss *et al.*, 1968). Interest has also centered on them because long-term administration is feasible. Thus it seems worthwhile to discuss in some detail the experimental observations obtained with these drugs.

a. Effects on Platelet Adherence and Release of Granule Contents from Adherent Platelets. Since platelet adherence and release of granule contents at an injury site are involved in both the initiation of thrombus formation when the subendothelium is exposed and the proliferation of smooth muscle cells at an injury site, considerable attention has been paid to the possibility of preventing the initial layer of platelets from adhering to the subendothelium. Although aspirin and sulfinpyrazone have been shown to inhibit adherence in a variety of *in vitro* test systems, it is now evident that they do not do so under physiologic conditions of flow, protein concentration, calcium concentration, and hematocrit (Weiss *et al.*, 1975; Tschopp, 1977; Cazenave *et al.*, 1978a,b; Kinlough-Rathbone *et al.*, 1980). Indomethacin appears to be partially inhibitory. All of these drugs, however, may limit the formation of a platelet aggregate on the adherent platelets by preventing thromboxane A_2 formation.

None of these drugs inhibits the release of granule contents from platelets that adhere to collagen or the subendothelium (Cazenave *et al.*, 1978b; Kinlough-Rathbone *et al.*, 1980). Thus, even in the presence of cyclooxygenase inhibitors, released ADP and serotonin could induce aggregate formation on the adherent platelets.

b. Inhibition of Vasoconstriction. Thromboxane A_2 is a potent vasoconstrictor (Ellis *et al.*, 1976; Needleman *et al.*, 1977b; Svensson and Fredholm, 1977) and has been implicated in coronary artery spasm (Ellis *et al.*, 1976). Increased concentrations of its product, thromboxane B_2, have been found in the peripheral blood of patients with vasotonic (Prinzmetal's) angina (Lewy *et al.*, 1979) and in the coronary sinus blood of patients with classical angina (Lewy *et al.*, 1980; Kuzuya *et al.*, 1980). Production of thromboxane A_2 has also been demonstrated during pacing-induced angina pectoris (Lewy *et al.*, 1980). Although aspirin and other nonsteroidal antiinflammatory drugs can block thromboxane A_2 formation, there is little evidence that they decrease the incidence of angina attacks. Aspirin does not improve exercise tolerance in patients with stable angina pectoris (Frishman *et al.*, 1976). Thromboxane formation may be a result rather than a cause of coronary artery spasm but it may, nevertheless, contribute to vasoconstriction.

It may be that platelet-rich thromboemboli lodge and persist in the microcirculation more readily when the thromboxane A_2 they produce causes vasoconstriction, than when thromboxane A_2 formation has been blocked by drug administration (Braunwald, 1978; Mustard and Packham, 1980). There is no information about this possibility in the

coronary circulation but it may be important in the cerebral circulation since aspirin does reduce the incidence of transient ischemic attacks that are generally considered to be mainly due to thromboembolism.

c. *Prevention of Ventricular Fibrillation.* Both aspirin and sulfinpyrazone have been shown to inhibit the formation of platelet aggregates and the occurrence of fatal arrhythmias in dogs with experimentally stenosed coronary arteries (Folts *et al.*, 1976; Folts and Beck, 1980). These drugs have also been observed to prevent ischemia-induced ventricular fibrillation in the myocardium of dogs and cats (Vik Mo, 1977, 1978; Moschos *et al.*, 1978, 1980; Kelliher *et al.*, 1980; Povalski *et al.*, 1980). These effects, however, may not be mediated through inhibition of platelet function.

d. *Effects on Experimentally Induced Thrombosis in Animals.* Many ways have been devised to induce thromboembolism in experimental animals. Vessel injury has been produced by mechanical, electrical, chemical, and laser methods in a number of different mammalian species, and the effects of drug administration on thrombus formation have been assessed. Several reviews of experiments of this type have been published (Didisheim, 1972; Didisheim and Fuster, 1978; Mustard and Packham, 1978, 1980; Philp, 1979). Reported effects of nonsteroidal antiinflammatory drugs range from inhibition, to "no effect," to enhancement of thrombosis. Enhancement has been attributed to inhibition of PGI_2 formation and has been demonstrated in injured veins under conditions of stasis (Kelton *et al.*, 1978a). In the microcirculation, inhibition of PGI_2 formation with agents that block the action of prostacyclin synthetase has been shown to enhance thrombosis (Bourgain, 1978, 1979, 1980; Rosenblum and El-Sabban, 1978), although in the same system aspirin decreased white platelet thrombus formation (Bourgain, 1978). In injured arteries, aspirin does not increase the number of platelets that adhere to the subendothelium, indicating that insufficient PGI_2 accumulates to affect platelet adherence when blood flow is rapid (Dejana *et al.*, 1980). It seems likely that the apparently contradictory reports in the literature concerning the effect of aspirin and other cyclooxygenase inhibitors on thrombosis in experimental animals may be due to differences in dosages, the types of vessels studied, blood flow, and perhaps the species under investigation.

e. *Duration of the Effects of Aspirin and the Question of Dosage.* The questions of the amount of aspirin that should be admin-

istered to inhibit thrombosis, and the frequency of administration, are not settled. It is well established that aspirin irreversibly acetylates cyclooxygenase and thus blocks its action on arachidonate (O'Brien *et al.*, 1970; Rome *et al.*, 1976; Burch and Majerus, 1979). Since platelets cannot synthesize new molecules of cyclooxygenase to replace those that have been inactivated, the effects of aspirin on a platelet persist for the remaining life of the platelet. Although inhibition of some platelet functions can be demonstrated for as long as 4–7 days after a single dose of aspirin (Weiss *et al.*, 1968; Zucker and Peterson, 1968; O'Brien *et al.*, 1970; Kocsis *et al.*, 1973), most aggregation responses are at least partially restored when as few as 10% normal platelets are present (O'Brien 1968; Valeri and Feingold, 1974; Cerskus *et al.*, 1980). A linear return of the ability of platelets to form malondialdehyde, a byproduct of prostaglandin synthesis, after aspirin ingestion was described by Stuart *et al.* (1975) and has been used as a method of determining platelet survival (although it actually assesses platelet production). Aspirin, however, can prevent isolated megakaryocytes from synthesizing prostaglandins (Demers *et al.*, 1977), presumably because it acetylates the cyclooxygenase and, if this occurs *in vivo* and the acetylated cyclooxygenase of the megakaryocytes is not replaced, one would expect that the entry of unaffected platelets into the circulation would be delayed. Burch *et al.* (1978a) have concluded that megakaryocyte cyclooxygenase is acetylated because the cyclooxygenase of human platelets, isolated up to 48 h after administration of 325 mg of aspirin, cannot be acetylated with radio-labeled aspirin. In support of this conclusion are the observations of some investigators of a lag phase of several days before the platelets from subjects who have ingested aspirin begin to regain their ability to synthesize malondialdehyde (Kocsis *et al.*, 1973; Leone *et al.*, 1979), and the report of Patrono *et al.* (1980) that single 100–400-mg doses of aspirin cause 94–98% inhibition of the ability of platelets to form thromboxane at 24 and 48 h. The situation has been complicated, however, by the recent report of Catalano *et al.* (1981) that platelet recovery from aspirin inhibition *in vivo* appears to be linear only if malondialdehyde and thromboxane B_2 production are measured in a protein-free medium. If these products are assayed in a protein-containing medium, a 48-h lag in recovery is seen. To accept the idea that aspirin irreversibly acetylates the cyclooxygenase of megakaryocytes, one has to assume that megakaryocytes are unable to synthesize the enzyme for a considerable time before they give rise to circulating platelets. The dosage of aspirin also affects the extent of acetylation of the cyclooxygenase of circulating platelets (Burch *et al.*, 1978a). Twenty-four hours after an oral dose of 160 mg, 82% of

the cyclooxygenase is acetylated, whereas after 650 mg more than 95% is acetylated.

In considering the effects of aspirin on platelets, megakaryocytes, and the vessel wall (see following paragraphs), it must be kept in mind that aspirin is rapidly hydrolyzed to salicylate, which has little or no effect on cyclooxygenase (Weiss *et al.*, 1968; O'Brien, 1968; Zucker and Peterson, 1970; Vargaftig, 1978). Hydrolysis occurs before and during absorption from the gastrointestinal tract, and in the circulation (Riegelman, 1971; Cohen, 1976). After an oral dose of 650 mg of aspirin, the peak concentration of about 120 m*M* occurs at approximately 20 min and by 90 min virtually all of the aspirin has been hydrolyzed (Rowland and Riegelman, 1968). It is the transient peak concentration in the blood that should be considered.

Although aspirin acetylates the cyclooxygenase of the cells of the vessel wall and thus prevents them from forming PGI_2 (Fig. 1), the inhibition is temporary because, in contrast to platelets, the cells are nucleated and able to synthesize new enzyme molecules. The length of time required for the cells of the vessel wall to regain their ability to synthesize PGI_2 seems to depend on the experimental approach that is used. Investigators who have studied this with endothelial cells in culture have reported times of 2 h (Czervionke *et al.*, 1979; Hoak *et al.*, 1980) and 36 h (Jaffe and Weksler, 1979). A recent report by Whiting *et al.* (1980) indicates that smooth muscle cells in culture regain their ability to synthesize PGI_2 from exogenous arachidonate in 1 to 2 h after aspirin treatment, whereas PGI_2 formation in response to thrombin is permanently impaired in nondividing cultures and only returns when the cells are subcultured and begin to divide. Hoak *et al.* (1980) also reported that endothelial cells in culture become unresponsive to thrombin and do not produce PGI_2 in response to a second stimulation with thrombin. In human subjects, Masotti *et al.* (1979) studied PGI_2 in venous blood following ischemia of the forearm caused by constriction; inhibition of PGI_2 formation by 2 to 5 mg/kg of aspirin was apparent immediately but had disappeared by 24 h. In experimental animals, differences in the length of time before the ability to synthesize PGI_2 is restored are probably due to variations in the dosage of aspirin, method of administration, species of animal, the type of vessel used, and the method of measuring prostacyclin (Villa and de Gaetano, 1977; Kelton *et al.*, 1978a; Buchanan *et al.*, 1979; Villa *et al.*, 1979; Ellis *et al.*, 1980). Regardless of the wide variations in reported times for the restoration of PGI_2 synthesis after aspirin administration, all tbe experimental evidence indicates that this does occur and is largely complete by 24 h. These observations concerning the persistence of the effect of aspirin

on platelets and the transient nature of its effect on PGI_2 production by the vessel wall have led to the suggestion that aspirin should be administered once daily (rather than more frequently) to minimize inhibition of PGI_2 formation. Indeed, some authors have recommended administration at 48- or 72-h intervals (Burch and Majerus, 1979; Masotti *et al.*, 1979; Wenger and Hull, 1980; Patrono *et al.*, 1980).

It must be emphasized that these proposals are based on the assumptions that PGI_2 formation by the vessel wall has a key role in controlling the extent of thrombosis, and that aspirin exerts no effect on the clinical complications of atherosclerosis other than through inhibition of cyclooxygenase. However, there is experimental and clinical evidence against the concept that inhibition of PGI_2 production promotes thrombosis in large arteries. Dejana *et al.* (1980) have demonstrated that inhibition of PGI_2 formation by aspirin administration to rabbits does not increase platelet accumulation on the endothelium or on the subendothelium of the aorta. In addition, there is no evidence that, in man, thrombosis is promoted by high doses of aspirin that would be expected to inhibit PGI_2 formation. Patients with rheumatoid arthritis receive high doses of aspirin over prolonged periods. Investigators at the Mayo Clinic (Linos *et al.*, 1978) who observed a group of 473 such patients over 10 yr found that the incidence of myocardial infarction, angina pectoris, sudden death, or cerebral infarction did not differ significantly from that of untreated normal individuals of the same age and sex in Rochester, Minnesota. An earlier study of patients with rheumatoid arthritis, who were treated with large doses of aspirin, demonstrated a lower than usual incidence of myocardial infarction as a cause of death (Davis and Engleman, 1974). Thus, in rheumatoid arthritics at least, high doses of aspirin do not appear to increase the incidence of the clinical complications of atherosclerosis.

An indication that aspirin may have effects other than acetylation of cyclooxygenase comes from the studies of McKenna *et al.* (1980) with patients undergoing knee surgery. Aspirin did not have a beneficial effect in doses (325 mg t.i.d.) that have been shown to inhibit thromboxane A_2 formation by platelets, but in high doses (1.3 g t.i.d.) it did significantly reduce the incidence of deep vein thrombosis. Moroz (1977) has shown that high doses of aspirin and sodium salicylate increase the fibrinolytic activity of whole blood. This may be one of the "other effects" of aspirin on thrombosis.

Controversy also surrounds the question of a suitable dose of aspirin. Several groups of investigators have reported that the cyclooxygenase of the cells of the vessel wall is less sensitive to inhibition by aspirin than the cyclooxygenase of platelets (Burch *et al.*, 1978a; Masotti *et al.*,

1979; Shaikh et *al.*, 1980; Ellis et *al.*, 1980). Consequently, the concept has arisen that thromboxane A_2 formation by platelets can be inhibited by low doses of aspirin that will have little effect on PGI_2 production by the vessel wall. This possibility seems to be attractive because of the prospect that the side effects of aspirin on gastrointestinal bleeding could be reduced if the dosage were lowered. Low dose aspirin (150 mg/day) does appear to inhibit the type of thrombosis that occurs in arteriovenous shunts (Harter et *al.*, 1979). Many recommendations have been published for low aspirin dosage (Burch et *al.*, 1978a; Masotti et *al.*, 1979; Patrono et *al.*, 1980; Ellis et *al.*, 1980; Huijgens et *al.*, 1980). It should be emphasized, however, that other workers have not demonstrated a difference between the susceptibility of platelet and vessel wall cyclooxygenase to inhibition by aspirin (Jaffe and Weksler, 1979), and recent direct observations indicate that even low doses of aspirin (150 or 300 mg) inhibit PGI_2 formation by human vein segments for as long as 8 h (Preston et *al.*, 1981).

In all of the clinical trials except that of Elwood et *al.* (1974), aspirin was given in doses of 1 to 1.5 g/day, in divided doses. These trials were initiated before the discovery of PGI_2 or of the fact that aspirin inhibits its formation. However, as Genton (1980) has pointed out, there is "no persuasive evidence that the dosage used in the various trials have been excessive to negate the beneficial effects or be thrombogenic."

Considering all the observations to date, it is probably fair to conclude that the amount of aspirin given or the frequency of its administration are receiving more attention than they deserve. It has always seemed illogical that those who promote low dose aspirin given once daily (or even less frequently) often do not specify dosage on a per kilogram basis, as is always done in experimental animals. A high dose for a 50-kg patient is a much lower dose for a 125-kg patient. Time of administration relative to meals should also be considered because of differences in the time available for hydrolysis of aspirin in the gastrointestinal tract. If the only objective is to prevent thromboxane A_2 formation by platelets in the hope that this will inhibit the contribution of platelets to some forms of thrombosis, then doses between 150 and 600 mg/day for a 70-kg patient probably provide a reasonable balance between this objective and the goal of minimizing gastrointestinal bleeding. This appears to be a more important balance to consider than that between inhibition of thromboxane A_2 formation and inhibition of PGI_2 formation by the vessel wall.

f. Comparison of the Effects of Aspirin and Sulfinpyrazone. Because of its ability to acetylate the cyclooxygenase of platelets and thus irre-

versibly inactivate it, the effects of aspirin differ from those of other drugs that inhibit this enzyme. The other nonsteroidal antiinflammatory drugs, and sulfinpyrazone, are only inhibitory during the time that effective concentrations of the drugs or their active metabolites remain in the circulation. The contrast between the results with aspirin and with sulfinpyrazone in the clinical trials has stimulated interest in the other effects that these drugs may have on the clinical complications of atherosclerosis.

In doses that are commonly used, aspirin inhibits PGI_2 formation by vessel walls (Burch *et al.*, 1978b; Masotti *et al.*, 1979) but sulfinpyrazone has practically no effect (Gordon and Pearson, 1978; Livio *et al.*, 1980). Aspirin is rapidly hydrolyzed to salicylate *in vivo* and this product does not inhibit cyclooxygenase (Weiss *et al.*, 1968; Vargaftig, 1978), although it may act in other ways. In contrast, some of the metabolic products of sulfinpyrazone have the same type of inhibitory effect on cyclooxygenase as the parent compound (Buchanan *et al.*, 1978; Pedersen and Jacobsen, 1979; Butler *et al.*, 1980; Livio *et al.*, 1980). One of the earliest findings with sulfinpyrazone was prolongation of the shortened platelet survival that is often observed in patients with atherosclerosis (Smythe *et al.*, 1965); this effect on platelet survival has been noted subsequently by other investigators (Genton and Steele, 1977). Steele and Rainwater (1980) have reported recently that the shortened platelet survival observed in patients with rheumatic heart disease (mitral stenosis) is prolonged by the administration of sulfinpyrazone and that the drug decreases the incidence of thromboembolism in these patients. In contrast, aspirin does not change platelet survival or turnover (Harker and Slichter, 1972). It has been suggested that the prolongation of shortened platelet survival may be related to the ability of sulfinpyrazone to lessen endothelial cell injury, because experimentally it has been shown to protect against such injury caused by the continuous infusion of homocysteine into baboons (Harker *et al.*, 1978). In contrast, aspirin does not provide protection from endothelial cell injury induced in this way.

Results from the clinical trials of aspirin and sulfinpyrazone in post myocardial infarction patients cannot be compared directly because the trial designs differed. In the first trial, sulfinpyrazone reduced the incidence of sudden death in the first 6 months after myocardial infarction (Anturane Reinfarction Trial, 1980) whereas in the second trial, sulfinpyrazone had no apparent effect on sudden death although it significantly reduced the incidence of reinfarction (Anturan Reinfarction Italian Study, 1982).

In the trials with aspirin, many patients were admitted months or

years after their myocardial infarction so it is not possible to determine whether aspirin decreases the incidence of sudden death in the early period. Retrospective analysis of the trial of Elwood *et al.*, (1974) in which the first patients to be enroled entered tbe trial shortly after their myocardial infarction shows a trend toward benefit from aspirin but it is not statistically significant because of tbe small numbers in this group of patients. The PARIS trial (Persantine-Aspirin Reinfarction Study Research Group, 1980) also showed a trend toward benefit from aspirin for the patients who entered the study soon after their myocardial infarction. In neither of these trials, however, was the benefit on sudden death, and thus the results differ from those of the first trial with sulfinpyrazone (Anturane Reinfarction Trial, 1980).

Aspirin and sulfinpyrazone were compared in the Canadian Cooperative Study (1978) of patients with transient attacks of cerebral ischemia but only aspirin was shown to reduce the incidence of stroke, death, and recurrent transient attacks. Since thromboembolism in the main arteries supplying the brain is probably the cause of the majority of the attacks of transient cerebral ischemia, it seems likely that aspirin, but not sulfinpyrazone, influences this type of thromboembolism. In contrast, both sulfinpyrazone and aspirin decrease thrombosis in arteriovenous shunts (Kaegi *et al.*, 1975; Harter *et al.*, 1979). There is a possibility that different mechanisms have major roles in thrombus formation in diseased arteries and at the site where a shunt joins a vein. It is unlikely that formation of thromboxane A_2, which would be inhibited by both drugs, is dominant in both mechanisms. Our present knowledge does not permit definitive conclusions about these points.

g. *Differences in Effects of Aspirin or Sulfinpyrazone in Males and Females.* Some of the clinical trials have indicated that aspirin may be of more benefit to male patients than to female patients with clinical complications of atherosclerosis (Canadian Cooperative Study Group, 1978; Linos *et al.*, 1978; Elwood and Sweetnam, 1979). This pattern has also been recognized in patients undergoing total hip replacement who are at risk of venous thromboembolism (Harris *et al.*, 1977). In experiments with rabbits, Kelton *et al.* (1978b) observed that, in injured veins, doses of aspirin that inhibited thrombus formation in males were ineffective in females.

Kaegi *et al.* (1975) reported that the reduction in thrombosis in arteriovenous shunts by sulfinpyrazone was more evident in male patients than in females, but the incidence in untreated females was considerably lower than in males. During drug administration, the incidence of thrombosis was similar in males and females. However,

Harter *et al.* (1979) observed no difference between males and females in their study of the effect of aspirin on thrombosis in arteriovenous shunts.

The reason for a difference in the response of males and females to these drugs is not known. It may be related to the well-known fact that before menopause women have a lower incidence of the clinical complications of atherosclerosis than men of the same age, possibly due to a protective effect of estrogen (Uzunova *et al.*, 1978; Wessler, 1980). In males, there may be a mechanism promoting thrombosis that is of less importance in females. If so, it could be theorized that this mechanism can be inhibited by aspirin and sulfinpyrazone.

B. Effects of Agents That Increase Cyclic AMP Concentration

Most platelet reactions that are involved in thrombus formation are inhibited when the cyclic AMP (cAMP) concentration in platelets is increased above the very low basal level (Haslam *et al.*, 1978; Mustard and Packham, 1978; Gerrard and White, 1978). The agents that stimulate adenylate cyclase so that platelet ATP is converted to cAMP cause large increases in cAMP, whereas the agents that inhibit the phosphodiesterases that convert cAMP to AMP have little effect by themselves on the concentration of cAMP (Haslam, 1973; Lam *et al.*, 1981) although they greatly augment the increase in cAMP caused by stimulation of adenylate cyclase.

Adenylate cyclase can be stimulated by the prostaglandins PGI_2, PGD_2, and PGE_1 (Gorman *et al.*, 1977; Tateson *et al.*, 1977; Mustard and Packham, 1978) and by adenosine (Haslam, 1973). PGI_2 is produced by the cells of the vessel wall in response to injury, thrombin, bradykinin, angiotensin II, and histamine (Weksler *et al.*, 1977a, 1978; Schrör *et al.*, 1977; Blumberg *et al.*, 1977; Czervionke *et al.*, 1979; Baenziger *et al.*, 1980). Small amounts of PGD_2 are formed by platelets from arachidonate (Smith *et al.*, 1974b). PGE_1 is not formed in detectable amounts by platelets or the cells of the vessel wall but many experiments have been done with this prostaglandin because it is relatively stable and was available for study many years before PGI_2 was discovered. PGE_1 and PGI_2 have been shown to interact with the same receptor on the platelet membrane (Schafer *et al.*, 1979), although PGE_1 is considerably less potent in raising cAMP levels. Adenosine is readily formed from ADP or ATP that may be freed from damaged cells or released from platelets,

because there are enzymes in plasma that convert the adenine nucleotides to adenosine (Holmsen and Holmsen, 1971).

The phosphodiesterase inhibitors include dipyridamole (persantine) and related pyrimido-pyrimidines such as RA 233, RA 433, VK 744, and VK 774, the methylxanthines (caffeine, theophylline, and others), and papaverine (Mustard and Packham, 1978). Of these, dipyridamole has been studied most extensively because it was already in use in man as a coronary vasodilator before its effects on platelet function were recognized.

When platelet cAMP is increased, platelet adherence to collagen and the subendothelium can be shown to be inhibited *in vitro* and *in vivo* (Cazenave *et al.*, 1978b,c, 1979; Higgs *et al.*, 1978; Kinlough-Rathbone *et al.*, 1978; Harker *et al.*, 1979; Groves *et al.*, 1982a). Platelet aggregation in response to all stimuli and the release of platelet granule contents are also inhibited when platelet cAMP concentrations are high (Mustard and Packham, 1978; Moncada and Vane, 1979). Inhibition of platelet shape change (Kinlough-Rathbone *et al.*, 1970) may be responsible for some of these effects and has also been shown to prevent platelet membrane sites involved in the intrinsic coagulation pathway from becoming available to accelerate the generation of thrombin (Ehrman and Jaffe, 1980; Bunting and Moncada, 1980).

PGE_1 and PGI_2 also prevent the binding of fibrinogen to platelets that normally occurs in response to stimulation with ADP, possibly by preventing the membrane receptors for fibrinogen from becoming available for the aggregation process (Mustard *et al.*, 1978; Hawiger *et al.*, 1980; Harfenist *et al.*, 1981).

In high doses, PGE_1 and PGI_2 are strong inhibitors *in vivo* of platelet aggregation and thrombus formation in experimental animals (Woods *et al.*, 1978; Coppe *et al.*, 1978; Aiken *et al.*, 1979, 1980; Rosenblum and El-Sabban, 1979; Bourgain, 1979; Ohlendorf *et al.*, 1980; Plachetka *et al.*, 1980).

In a variety of clinical situations, PGE_1 and PGI_2 have been shown to have a strong inhibitory effect on platelet adherence, thrombosis, and thromboembolism. These prostaglandins prevent platelet loss during extracorporeal circulation (Addonizio *et al.*, 1979; Longmore *et al.*, 1979; Rådegran and Papaconstantinou, 1980; Walker *et al.*, 1981) and charcoal hemoperfusion (Bunting *et al.*, 1979; Langley *et al.*, 1979; Gimson *et al.*, 1980). Beneficial effects have also been reported in advanced atherosclerosis of the peripheral circulation (Carlson and Olsson, 1976; Szczeklik *et al.*, 1979, 1980a; Budd *et al.*, 1980), and in kidney transplant rejection (Leithner *et al.*, 1981).

Both PGE_1 and PGI_2 have the side effects of increased heart rate,

hypotension, facial flushing, and headache so their use has usually been limited to short-term administration (Carlson *et al.*, 1968; Gryglewski *et al.*, 1978a; Szczeklik *et al.*, 1978; O'Grady *et al.*, 1980). Under *in vivo* conditions, PGI_2 is rapidly converted to its relatively inactive product, 6-keto-$PGF_{1\alpha}$ (the half-life of PGI_2 is 2–3 min), so it is usually given by continuous infusion and its effects on the circulation quickly disappear when the infusion is stopped.

In experimental animals, both PGE_1 and PGI_2 have been observed to preserve ischemic myocardial tissue (Takano *et al.*, 1977; Ogletree and Lefer, 1978; Lefer *et al.*, 1978; Araki and Lefer, 1980; Au *et al.*, 1980). In ischemic canine myocardium, however, infusion of PGI_2 did not affect transmural or regional blood flow (Einzig *et al.*, 1980). PGI_2 did not decrease the incidence of occlusion-induced arrhythmias in rats (Au *et al.*, 1980).

One short report by Szczeklik *et al.* (1980b) indicates that PGI_2 infusion for 12 to 48 h may lessen the incidence of spontaneous angina attacks in some patients for at least four weeks. In contrast, Chierchia *et al.* (1982) reported that PGI_2 was ineffective in reducing the incidence, severity or duration of ischemic episodes due to coronary vasospasm in patients with variant angina.

1. PGI_2 AND ATHEROSCLEROSIS. The possibility that less PGI_2 is synthesized by atherosclerotic vessels so that platelet adhesion and thrombus formation are not inhibited has aroused interest. Some investigators have reported that PGI_2 synthesis is decreased in human atherosclerotic plaques (D'Angelo *et al.*, 1978; Sinzinger *et al.* (1979a). Although depressed PGI_2 synthesis was observed by Gryglewski *et al.*, (1978b) in vessels from rabbits fed an atherogenic diet, Sinzinger *et al.* (1979b) found increased synthesis by arterial tissue from mini-pigs fed an atherogenic diet. The latter group have suggested, however, that the susceptibility of various species to atherosclerosis is negatively correlated with the amount of PGI_2 synthesized by vascular tissue (Sinzinger *et al.*, 1980).

Blood vessels from diabetic patients (Silberbauer *et al.*, 1979; Johnson *et al.*, 1979) and from animals with experimentally induced diabetes (Harrison *et al.*, 1978; Gerrard *et al.*, 1980; Subbiah and Deitemeyer, 1980; Carreras *et al.*, 1980; Silberbauer *et al.*, 1980) synthesize less PGI_2 than vessels from normal individuals and animals. These observations have been linked to the predisposition of diabetic patients to atherosclerosis and its clinical complications. However, Davis *et al.* (1979) have determined that the circulating concentrations of 6-keto-$PGF_{1\alpha}$, the stable product formed from PGI_2, are similar in diabetic

and nondiabetic subjects. It may be that the local concentration of PGI_2 at an injury site on a vessel wall has more effect on platelet adherence and the initiation of atherosclerotic lesions than the low concentrations in the circulation.

Controversy surrounds the suggestion that, under normal conditions, PGI_2 may be a circulating hormone, generated in the lungs, that inhibits platelet aggregation *in vivo* (Gryglewski *et al.*, 1978c; Moncada *et al.*, 1978a). This suggestion was made on the basis of studies with 15-hydroperoxyarachidonic acid, an inhibitor of PGI_2 synthetase, and with an antibody directed against a stable PGI_2 analog. Other investigators, however, were unable to demonstrate that addition of the antibody to depress circulating PGI_2 levels affected blood pressure in the absence of exogenous PGI_2, or changed the extent of ADP-induced platelet aggregation in platelet-rich plasma prepared and tested within 3 min of blood sampling (Smith *et al.*, 1978; Steer *et al.*, 1980). Several groups have found that the concentration of PGI_2 in the circulation of man and animals under normal conditions is too low to affect platelet aggregation or adherence (Fitzgerald *et al.*, 1981; Haslam and McClenaghan, 1981; Christ-Hazelhof and Nugteren, 1981).

Attempts are being made to synthesize active analogs of PGI_2 that are more stable under *in vivo* conditions (van Dorp *et al.*, 1978; Blaskó *et al.*, 1980; Whittle *et al.*, 1980; Karim and Adaikan, 1980; Morita *et al.*, 1980; Karim *et al.*, 1981; Schrör *et al.*, 1981.

2. Effects of Dipyridamole. Although dipyridamole by itself is a relatively weak inhibitor of platelet reactions *in vitro* and has little or no effect on the concentration of cAMP in platelets, it greatly augments the increase in cAMP caused by the prostaglandins that stimulate adenylate cyclase, and thus potentiates their inhibitory effects (Moncada and Korbut, 1978; Best *et al.*, 1979; Jørgensen *et al.*, 1979). Moncada and Korbut (1978) and Gryglewski *et al.* (1978d) have suggested that, *in vivo*, dipyridamole and other agents that inhibit phosphodiesterase may potentiate the inhibitory effect of any PGI_2 that may be formed. In view of the well-established *in vitro* findings, it is difficult to understand the report of Di Minno *et al.* (1979) that the administration of dipyridamole to human subjects diminisbed the inhibitory effect of PGI_2 on platelet aggregation, tested *in vitro*.

It has been reported recently that dipyridamole stimulates PGI_2 synthesis from PGH_2 by aortic microsomes (Blass *et al.*, 1980). Such an effect would further enhance the more-than-additive stimulation of cAMP formation caused by the combination of PGI_2 and dipyridamole.

Dipyridamole may have other effects that are relevant to thrombosis

and atherosclerosis in addition to its ability to raise cAMP in platelets stimulated with PGI_2. Some of its beneficial *in vivo* effects may be due to the vasodilation it causes, rather than to inhibition of platelet adherence and aggregation.

Although dipyridamole can be shown to inhibit thromboxane synthetase in isolated platelet microsome preparations (Greenwald *et al.*, 1978; Best *et al.*, 1979), Moncada *et al.* (1978b) observed that dipyridamole (200 μM) does not inhibit thromboxane A_2 formation from prostaglandin H_2 by intact platelets. Dipyridamole does not inhibit cyclooxygenase (Best *et al.*, 1979). Di Minno *et al.* (1978) have pointed out that the concentration of dipyridamole required to inhibit aggregation in human platelet-rich plasma, induced by a threshold concentration of arachidonate, is 50 μM whereas the therapeutic range is 1–8 μM, and less than 0.6 μM potentiates the inhibitory effect of PGI_2. They conclude that dipyridamole alone, in the concentrations that are achieved in man, is unlikely to affect platelet aggregation except by potentiating the effect of PGI_2.

Some of the pyrimido-pyrimidine drugs have been shown to diminish the extent of experimentally induced thrombosis in animals and in extracorporeal shunts (Mustard and Packham, 1978). Dipyridamole inhibits platelet adherence to collagen, to the subendothelium in rabbits and baboons, and to artifical surfaces, but the doses required are high (Cazenave *et al.*, 1978b; Kinlough-Rathbone *et al.*, 1978; Harker *et al.*, 1979; Groves *et al.*, 1982a). In baboons, in which atherosclerosis was induced by continuous infusion of homocysteine, dipyridamole diminished the extent of atherosclerosis (Harker, 1980), but enhanced formation of atherosclerotic plaques has been observed in rabbits fed an atherogenic diet and given dipyridamole (Dembińska-Kièć *et al.*, 1979). It is not known whether the differences in species and in the methods of inducing atherosclerotic lesions account for these contradictory results. In dogs and rhesus monkeys, the combination of dipyridamole and aspirin has been shown to diminish the extent of intimal thickening in vein grafts (Metke *et al.*, 1979; McCann *et al.*, 1980). In neither of these studies, however, were the individual drugs tested.

In man, shortened platelet survival is prolonged by dipyridamole or by the combination of dipyridamole and aspirin (Harker and Slichter, 1972; Steele *et al.*, 1978). Dipyridamole by itself has not been reported to be helpful in managing arterial or venous thrombosis (Verstraete, 1978; Mustard and Packham, 1978), although combination with aspirin or an anticoagulant appears to be effective in some cases. In a variety of thromboembolic conditions in small vessels in man, the combination of dipyridamole and aspirin seems to be beneficial (Zacharski *et al.*,

1971; Giromini *et al.*, 1972; Amir and Krauss, 1973; Abrahamsen *et al.*, 1974; Wu and Hoak, 1976).

3. Effects of Dipyridamole Combined with Aspirin. The combination of dipyridamole and aspirin had been tested by a number of investigators before PGI_2 and the effect of aspirin on its production by vessel walls were recognized. If the theory that dipyridamole potentiates the effect of PGI_2 *in vivo* is valid, then administration of aspirin in doses sufficient to inhibit PGI_2 formation should reduce the inhibitory effect of dipyridamole. In support of this hypothesis, Moncada and Korbut (1978) have reported that high doses of aspirin completely inhibited the deaggregating effect of dipyridamole on platelets that had aggregated on tendons superfused with rabbit blood, whereas low doses of aspirin (which are thought to inhibit only thromboxane A_2 formation) potentiated the deaggregating effect of dipyridamole. Honour *et al.* (1977) observed a synergistic inhibitory effect of dipyridamole and aspirin on ADP-induced thrombosis in rabbits, and pointed out that, on a per kilogram basis, the doses were similar to those in the Persantine-Aspirin Reinfarction Study (1980). However, in this trial, no statistically significant difference was found between the results with aspirin alone, and the combination of aspirin and dipyridamole, although a trend toward increased benefit for the combination was observed at some time points.

C. Drugs That Act through Other Mechanisms

The list of drugs and compounds that have been shown to inhibit platelet function *in vitro* is long (Mustard and Packham, 1978). Some of these agents have been administered to experimental animals and shown to affect platelet reactions *in vivo* and *ex vivo*. A few have been tested after administration to man, but for several of these, such as propranolol and other β-blockers, and clofibrate and halofenate, their inhibition of platelet function is incidental to their other effects. In many cases, the mechanisms of action of these heterogeneous drugs are unknown.

1. Propranolol and Other β-Blockers. Although propranolol and other β-adrenoceptor blocking drugs inhibit platelet responses that depend on phospholipase A_2 activation (Frishman *et al.*, 1974; Weksler *et*

al., 1977b; Hawiger and White, 1978; Vanderhoek and Feinstein, 1979; Vlachakis and Aledort, 1980), their major effects involve reduction in heart rate and myocardial contractility and hence in oxygen requirements. These drugs reduce pain and improve exercise tolerance in patients with angina pectoris. The clinical pharmacology has been reviewed recently (Frishman, 1980) in relation to primary prevention of myocardial infarction and sudden death in these patients, treatment in acute myocardial infarction, and antiarrhythmic effects. In these primary prevention trials, the drugs did not increase survival, but some of the postmyocardial infarction trials have shown these drugs to reduce the incidence of sudden death and reinfarction (Wilhelmsson *et al.*, 1974; Ahlmark *et al.*, 1974; Norris *et al.*, 1978; Andersen *et al.*, 1979; Norwegian Multicenter Study Group, 1981); although others have not shown statistically significant effects (Wilcox *et al.*, 1980; Baber *et al.*, 1980), the recently reported β-Blocker Heart Attack Trail (1982) of 3837 post-myocardial infarction patients showed a beneficial effect of propranolol on total mortality, arteriosclerotic heart disease mortality and sudden death. It should be noted that in the large-scale clinical trials of aspirin and sulfinpyrazone some of the patients were also receiving propranolol and this was only considered in retrospective subgroup analysis.

2. NITROGLYCERIN AND NITROPRUSSIDE. Nitroglycerin and other organic nitrate vasodilators are commonly used to treat angina pectoris. Nitoglycerin, in higher concentrations than those reached *in vivo*, has recently been reported to block both primary and secondary platelet aggregation induced by ADP *in vitro*, and to inhibit aggregation in response to collagen, epinephrine, arachidonate, and the ionophore A23187 (Pfister and Imhof, 1979; Schafer *et al.*, 1980). Arachidonate oxygenation was also inhibited by nitroglycerin at a concentration of 25 μ*M*. Although Levin *et al.* (1981) showed that nitroglycerin induces PGI_2 formation by endothelial cells in culture, there is no evidence that it does so *in vivo*.

Sodium nitroprusside inhibits platelet aggregation at the concentrations that are achieved by therapeutic doses (Pfister and Imhof, 1979). It is not known whether part of the beneficial effects of these drugs in coronary artery disease may be due to inhibition of platelet aggregation and thromboxane A_2 formation.

3. SULOCTIDIL. Suloctidil is a vascular antispasmodic drug that also inhibits platelet aggregation (Roba *et al.*, 1976; de Gaetano *et al.*, 1976; Stelzer *et al.*, 1979) and depletes platelets of serotonin (Mills and Mac-

farlane, 1976; de Gaetano *et al.*, 1977). Suloctidil does not appear to affect the arachidonate pathway in platelets. It can be administered to man in doses that inhibit platelet aggregation, tested in citrated platelet-rich plasma. In low concentrations it appears to stabilize plasma membranes and prevent hemolysis, whereas at higher concentrations it disrupts membranes and causes cell lysis (Mürer *et al.*, 1979). Antithrombotic effects have been demonstrated in experimental animals (Gurwich and Lipinski, 1976; Stelzer *et al.*, 1979). Suloctidil has been found to prolong shortened platelet survival after cardiac valve replacement (Col-de Beys *et al.*, 1981). The initiation of a clinical trial of this drug in patients after cerebral infarction has been reported (Passamani, 1980) with myocardial infarction as a secondary endpoint.

4. Clofibrate and Halofenate. Clofibrate was administered to patients to lower plasma lipid concentrations before its effects on platelets were recognized. Clofibrate and halofenate inhibit platelet responses that are largely dependent on thromboxane A_2 formation (Robinson and Le Beau, 1967; Lin and Smith, 1976; Favis and Colman, 1978) and decrease platelet hypersensitivity in patients with familial hyperbetalipoproteinemia (Carvalho *et al.*, 1974; Colman *et al.*, 1976; Colman, 1978). The abnormally high cholesterol–phospholipid ratio in the platelet membrane of patients with type IIa hyperbetalipoproteinemia is decreased by these drugs (Colman, 1978). This ratio affects membrane fluidity and hence may control the availability of membrane enzymes and receptors.

Clofibrate prolongs shortened platelet survival (Gilbert and Mustard, 1963; Glynn *et al.*, 1967; Steele *et al.*, 1975; Harker and Hazzard, 1977) but has been reported to decrease PGI_2 synthesis in rabbit aortae and in human forearm veins (Sinziger *et al.*, 1979c).

Although a lower incidence of nonfatal myocardial infarction was observed in patients with a history of angina or high serum cholesterol concentrations who were treated with clofibrate (Five-Year Study, 1971; Oliver, 1971), the incidence of fatal heart attacks was unchanged (Committee of Principal Investigators, 1978). In a secondary prevention trial with patients who had had a myocardial infarct, clofibrate did not decrease mortality (Coronary Drug Project Research Group, 1975). Recently, the committee of principal investigators (Oliver *et al.*, 1980) of the W.H.O. Cooperative Trial on primary prevention of ischemic heart disease reported the results of the mortality follow-up of men treated with clofibrate for a mean time of 5.3 yr who had been observed for 4.3 yr after the treatment was discontinued (a total of 9.6 yr of observation). There were 25% more deaths from all causes in the clofibrate-treated

group than in the comparable, high serum cholesterol, control group ($p < 0.01$). The explanation is not apparent. A long-term toxic effect of clofibrate or the consequences of reducing cholesterol pools were suggested. The inhibitory effect of clofibrate on platelet function becomes irrelevant in the light of its recently recognized harmful effect.

5. TICLOPIDINE [5-(*o*-CHLOROBENZYL)-4,5,6,7-TETRAHYDROTHIENO[3,2-C] PYRIDINE HYDROCHLORIDE]. Both the primary and secondary phases of ADP-induced platelet aggregation are inhibited in citrated platelet-rich plasma prepared from subjects who have ingested ticlopidine; aggregation and release in response to collagen, epinephrine, arachidonic acid, and thrombin are also decreased (O'Brien *et al.*, 1978; David *et al.*, 1979; Knudsen and Gormsen, 1979; Lips *et al.*, 1980; Nunn and Lindsay, 1980; Conard *et al.*, 1980; Kirstein *et al.*, 1980). Although some of these investigators were unable to demonstrate some of the inhibitory effects, the differences in the reports are probably attributable to dosage, time of blood sampling, and the concentration of aggregating agent used for the *in vitro* tests. Enhanced deaggregation has been observed. Ticlopidine is only weakly inhibitory if it is added directly to platelet-rich plasma and its effects reach a maximum about 24 h after administration to normal control subjects (O'Brien *et al.*, 1978; David *et al.*, 1979; Knudsen and Gormsen, 1979). Inhibition persists for several days. Ticlopidine has been shown to enhance the inhibitory effect of PGI_2 and PGE_1 on rat platelets (Johnson and Heywood, 1979; Ashida and Abiko, 1979) but the precise mechanism through which it (or its metabolite) exerts its inhibitory effects has not been identified.

Administration of this drug prolongs the bleeding time of human volunteers (David *et al.*, 1979; Lips *et al.*, 1980) and prolongs shortened platelet survival in rats (Wilkinson *et al.*, 1979). Inhibition of intimal proliferation in deendothelialized rabbit aortae has been reported by Reece and Walton (1979). Although a protective effect on experimentally induced peripheral arterial occlusive disease in rats was observed by Ashida *et al.* (1980), in 12 patients with peripheral atherosclerosis, ticlopidine did not produce significant changes in circulation parameters or walking distance (Kirstein *et al.*, 1980). Ticlopidine has been reported to inhibit thrombotic obstruction of arteriovenous shunts or vascular grafts in uremic patients under chronic hemodialysis (Kobayashi *et al.*, 1980) and to decrease platelet consumption during and after extracorporeal circulation in patients who underwent open-heart surgery for implantation of a valvular prosthesis (Installe *et al.*, 1981).

6. VITAMIN E. Vitamin E is an antioxidant that inhibits both platelet cyclooxygenase (Ali *et al.*, 1980) and lipoxygenase (Gwebu *et al.*, 1980). Its inhibitory effect on platelet aggregation has been attributed by some investigators to inhibition of thromboxane A_2 formation (Kurokawa *et al.*, 1971; Fong, 1976; Steiner and Anastasi, 1976). Others have suggested that it may directly alter membrane function (Cox *et al.*, 1980). A metabolite of vitamin E, vitamin E quinone, is a stronger inhibitor and prevents the mobilization of arachidonate as well as slightly inhibiting cyclooxygenase (Cox *et al.*, 1980). Both vitamin E and its quinone affect calcium flux in platelets (Butler *et al.*, 1979; Cox et al., 1980). Most of the studies of the effect of vitamin E on platelet function have been done *in vitro*. The *ex vivo* study of Gomes *et al.* (1976), in which α-tocopherol acetate was administered to patients with coronary artery disease and a matched control group for 8 days, showed no major effect of vitamin E on platelet aggregation in response to ADP or epinephrine. Lake *et al.* (1977) have reported that the enhanced platelet responsiveness to aggregating agents associated with vitamin E deficiency in infants is diminished by vitamin E administration. The claims that vitamin E is of benefit in preventing clinical complications of atherosclerosis have not been tested in large-scale, randomized, double-blind trials.

7. OTHER DRUGS. Many other drugs that are administered to man inhibit platelet functions (Packham and Mustard, 1977, 1980; Mustard and Packham, 1978). Among these drugs are penicillin and related antibiotics; antihistamine and antiserotonin drugs such as cyproheptadine, promethazine, and lidoflazine; the diuretic furosemide; imipramine, amitriptyline, and chlorpromazine; nitrofurantoin, glyceryl guaiacolate (in cough syrups); pyridinol carbamate; alcohol; pyridoxal 5′-phosphate (vitamin B_6) (Subbarao *et al.*, 1979; Kornecki and Feinberg, 1980); the α-adrenergic receptor blocking agents phentolamine and dihydroergotamine; nicergoline (which has α-blocking adrenolytic activity) (Praga *et al.*, 1979; Lagarde *et al.*, 1980); gliclazide (a hypoglycemic agent)(Ponari *et al.*, 1979); and piracetam (Henry *et al.*, 1978; Bick, 1979).

D. Drugs That Prevent Thrombin Formation or Its Activity

Thrombin causes platelet aggregation and release of granule contents and converts fibrinogen to fibrin. Thus thrombin may play an impor-

tant part in thrombus formation and stability. Many of the drugs that inhibit aggregation induced by collagen or arachidonate are ineffective against thrombin, notably the nonsteroidal antiinflammatory drugs. Although some drugs do inhibit thrombin-induced platelet aggregation (such as those that raise the cAMP concentration in platelets), the concentrations required for inhibition are generally much higher than the concentrations that inhibit aggregation induced by ADP, arachidonate, or collagen, and usually higher than can be achieved *in vivo* in man.

The effects of thrombin can be prevented by the administration of oral anticoagulants, such as the coumarin compounds to block thrombin formation, or by administration of heparin to block the action of thrombin and other activated factors of the intrinsic coagulation pathway such as factor Xa.

1. ORAL ANTICOAGULANTS. Although early studies failed to show a beneficial effect of oral anticoagulants on mortality in the acute phase of myocardial infarction (Wasserman *et al.*, 1966; Report of the Working Party on Anticoagulant Therapy in Coronary Thrombosis, 1969), more recent investigations have provided evidence favoring their use in the acute phase (Modan *et al.*, 1975; Tonascia *et al.*, 1975; Chalmers *et al.*, 1977; Szklo *et al.*, 1979; Sixty Plus Reinfarction Study Research Group, 1980). However, in the German–Austrian study of secondary prevention of myocardial infarction in which aspirin, phenprocoumon, and placebo were compared, phenprocoumon was no more effective than the placebo in preventing coronary deaths or events (Breddin *et al.*, 1980). The beneficial effects in the acute phase are likely due to reduction in the frequency or extent of thrombosis, but this may be in the venous as well as in the coronary circulation.

2. HEPARIN. In combination with antithrombin III, heparin is a powerful inhibitor of thrombin and other activated coagulation factors (Rosenberg, 1978; Wessler and Gital, 1979). Heparin blocks the effects of thrombin on platelets, but its effects on aggregation and release of granule contents induced by other aggregating agents range from potentiation to inhibition (Rowsell *et al.*, 1967; O'Brien *et al.*, 1969; Salzman *et al.*, 1980). Heparin may itself cause platelet aggregation (Zucker, 1975; Salzman *et al.*, 1980). Considerable variability has been noted among different commercially available heparin preparations and fractions of heparin, with respect to affinity for antithrombin III and effects on platelets (Wessler and Gitel, 1979; Salzman *et al.*, 1980). As much as two-thirds of some commercial heparin preparations have little or no

anticoagulant activity. Salzman *et al.* (1980) observed that the low molecular weight fractions of a porcine intestinal mucosal heparin they studied showed an inverse relationship between their platelet-aggregating effect and anticoagulant activity. Fractions of high molecular weight, (~20,000) were more reactive with platelets than the low molecular weight fractions (~7000). Thus it may be possible to isolate heparin fractions of low molecular weight and high antithrombin III affinity for antithrombotic therapy, that have little effect on platelets.

Althougb heparin has primarily been used to prevent venous thrombosis, it has been found to be as inhibitory as any of the other antithrombotic agents in experimentally induced arterial thrombosis and in extracorporeal shunts connected to animals (Mustard *et al.*, 1963; Didisheim, 1972; Mustard *et al.*, 1972; Philp *et al.*, 1978).

Heparin reduces the extent of smooth muscle cell proliferation after vessel wall injury (Clowes and Karnovsky, 1977; Guyton *et al.*, 1980), but this is probably through a direct effect on the smooth muscle cells rather than on platelets (Hoover *et al.*, 1980). Both anticoagulant and nonanticoagulant fractions are effective. Engleberg (1980) has recently reviewed the experimental and clinical evidence regarding heparin therapy in coronary artery disease and concluded that heparin reduces atherosclerosis in most studies of cholesterol-fed animals and that, although the evidence is controversial, long-term intermittent heparin therapy significantly decreases cardiovascular mortality in patients who begin heparin therapy at least 1 yr after myocardial infarction.

The effects of combining heparin with a prostaglandin that raises the cAMP concentration in platelets have been investigated *in vitro*. The results of this combination are complex. The potentially beneficial aspect is that these prostaglandins inhibit platelet shape change in response to aggregating agents (Ehrman and Jaffe, 1980) and thus prevent exposure of the membrane sites to which factor Xa binds. Unbound factor Xa is readily inhibited by the antithrombin-III–heparin complex (Miletich *et al.*, 1978) and hence these prostaglandins would be expected to enhance the inhibitory effect of heparin on coagulation. Bunting and Moncada (1980) have recently demonstrated that this occurs. The undesirable aspect of combining heparin with these prostaglandins is that heparin apparently inhibits the activation of adenylate cyclase by PGE_1 or PGI_2 (Saba *et al.*, 1979; Reches *et al.*, 1979; Eldor and Weksler, 1979), thus lessening the effect of these prostaglandins on platelet function (Eldor and Weksler, 1979). Theoretically, the combination of heparin with these prostaglandins should prevent the formation of thrombi if thrombin has a major role, but be less useful if platelet aggregation induced by collagen, ADP, and thromboxane A_2 is

dominant and PGI_2 acts in a regulatory way. It has been reported recently that heparin does not modify the prostacyclin-induced relaxation of the coronary vasculature (Eldor *et al.*, 1980). Study of the interaction of various heparin fractions and PGI_2 would seem to be warranted.

E. Combination of Drugs

Many investigators have reasoned that beneficial effects should be obtained from combinations of drugs that inhibit different platelet reactions (e.g., aspirin and dipyridamole), or combinations of drugs that inhibit platelet reactions with those that prevent thrombin formation or its actions (Packham and Mustard, 1977; Verstraete, 1978). Although no large clinical trials have been undertaken in which a drug that inhibits platelet reactions has been combined with heparin or an oral anticoagulant, it seems probable that such a combination would inhibit some types of arterial thrombosis. The bleeding problems that would be likely to occur lessen the feasibility of this approach. As mentioned earlier, the combination of aspirin with dipyridamole appears to be beneficial in conditions involving thrombosis in small vessels, and has been used in the Persantine-Aspirin Reinfarction Trial (1980).

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5 Platelets, Thrombosis, Atherosclerosis in Myocardial Infarction: Interactions and Relationships

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Myocardial Infarction and Cardiac Death

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I. INTRODUCTION

Concepts of the relationships of platelets and thrombosis to the pathogenesis of myocardial infarction and sudden death have recently undergone a paradoxical change. Whereas it used to be accepted that coronary thrombosis caused myocardial infarction and that the thrombotic process had little, if anything, to do with the initiation of atherosclerosis, both of these views have been challenged. In this chapter evidence will be presented that platelets play a key part at all stages of the development and progression of the disease. Although the coronary circulation may rarely be compromised by various inflammatory diseases of the arteries or by emboli arising in the left chambers of the heart or even more rarely by paradoxical embolism from thrombi arising in the systemic venous system, the usual disease leading to myocardial ischemia is atherosclerosis of the coronary arteries. Accordingly, in understanding the development of atherosclerosis, some exploration of the involvement of platelets and thrombosis in atherogenesis is relevant.

II. PLATELETS AND THROMBOSIS IN ATHEROGENESIS

A. Endothelial Injury

The possibility that the initial step in the atherosclerotic process may involve platelet interactions with the arterial wall has received some impetus from the recent revival of an old idea that the disease is essentially a reaction of the vessel wall to injury (Virchow *et al.*, 1856). Support for this idea comes from *in vivo* studies of the reaction of the vessel wall to injury (Moore, 1974, 1979) and *in vitro* studies employing arterial smooth muscle (Ross *et al.*, 1974) and endothelial cells (Thorgiersen and Robertson, 1978) in tissue culture. It has been known for some time that the synergy of hypercholesterolemia and any of a variety of forms of injury cause lipid-rich intimal thickenings (Minick, 1981). The combination of immunologically induced damage to the vessel wall and modest hyperlipemia is particularly effective in producing lesions that closely mimic or are identical to those of human

atherosclerosis (Minick *et al.*, 1966). There are a number of settings in which vascular injury in man results in the development of arteriosclerotic lesions (Moore and Ihnatowycz, 1978). These include the vessel lesions seen in homocystinemia (Harker *et al.*, 1974), the lesions induced in the aorta of neonates by the placement of arterial catheters to monitor blood gases (Tyson *et al.*, 1976), and the lipid-rich plaques observed in veins distal to an arteriovenous shunt (Stebbens and Karmody, 1975). In all of these settings continued or repeated endothelial or intimal injury provides the conditions necessary for the development of a proliferation of smooth muscle cells in the intima which then serves as a substrate for lipid deposition.

In considering the part played by platelets and thrombosis in the development of such lesions it is instructive to consider two broad categories of experimental injuries that can induce lesions that resemble the most prevalent forms of atherosclerotic lesions encountered in the arteries of man. One of these lesions is induced by continued or repeated mechanical (Moore, 1973) or immunological injury (Friedman *et al.*, 1975) to the intima of the aorta or carotid arteries of rabbits and has a close resemblance to the complicated lesions of human atherosclerosis. The other lesion is induced by removal of only the endothelial layer of the aorta using a Fogarty balloon catheter and resembles the human fibrous plaque (Moore, 1978).

1. Repeated Injury. The lesion developing in response to continued or repeated intimal injury was first observed in response to the intraaortic placement of a polyethylene catheter in the aortas of rabbits as a part of an experiment related to the production of focal ischemic lesions of the renal cortex by emboli of platelet or platelet-fibrin thrombi (Moore and Mersereau, 1965a,b). It was found that raised yellow lesions occurred at sites where it appeared that there might be repeated contact of the catheter with the vessel wall. In other areas, intimal thickenings appearing as grey opacities were present. A third type of lesion consisted of lipid-filled cells in the intima and resembled a fatty streak as observed in man. The occurrence of these lesions in a situation where mechanical injury and thrombosis was occurring in rabbits fed a normal diet indicated that hyperlipemia was not necessary for lipid-rich lesions to occur (Moore, 1973). However, for lipid to persist in the lesions it seemed that repeated or continued injury was needed.

This was tested further in a setting where repeated injury to the intima of a defined segment of an artery was immunologically induced. A segment of rabbit carotid artery, isolated between bulldog clamps,

was evacuated of blood and 0.3 ml of human serum which was cytotoxic for rabbit lymphocytes was placed in the vessel lumen for 3 min, then aspirated and blood flow reestablished. This was repeated weekly for 4 weeks. Five weeks following the first exposure (1 week following the last exposure) to lymphocytotoxic human serum raised lipid-containing lesions were found. Two or three injections induced fatty streaks or oedematous intimal thickenings. Raised lipid containing lesions also frequently showed calcification. Examination of the early stage of lesion development showed loss of endothelium 1 min after reestablishment of blood flow, rapidly followed by the deposition of platelet-fibrin thrombus (Friedman *et al.*, 1975).

An important characteristic of these injury-induced raised lesions is the tendency to regress when the injury stimulus is removed. The regression involved a decrease in size and an eventual loss of stainable lipid (Friedman *et al.*, 1976; Moore *et al.*, 1977). Regression also seemed to involve a transition from raised lipid containing lesions to fatty streaks. The fatty streaks were of short duration and seemed to evolve to nonlipid-containing fibromusculoeslastic plaques.

2. A Single Injury. Injury designed to remove only the endothelial layer also results in the development of a lesion that has some similarities but many significant differences from the lesions induced by repeated mechanical or immunological injury to the vessels.

Again, there is initial involvement of platelets in the process. The first morphological observation following balloon removal of the endothelium reveal platelets initially in small clusters, but within minutes as a monolayer spread out over the subendothelial connective tissue (Baumgartner and Studer, 1963; Baumgartner, 1972). There may also be transient association of leukocytes with the vessel wall (Ratliff *et al.*, 1979). Transmission electron microscopy shows that these are monocytes and neutrophil leukocytes. Smooth muscle cells appear on the luminal side of the internal elastic lamina and rapidly proliferate, forming eventually a neointima up to 15 to 20 cell layers thick (Stemerman and Ross, 1972). The platelet interaction with the vessel wall appears to be short-lived. Studies with ^{51}Cr-labeled platelets show an initial high uptake of platelets with little further uptake thereafter (Groves *et al.*, 1979). At 24 to 48 h following injury, platelets that were adherent to the surface are lost and by 7 days following injury very few remain. Little is known concerning the mechanism by which subendothelial tissues and the forming neointima become nonreactive to platelets.

The first step in the development of intimal thickening following

stripping of the endothelial layer appears to be migration of smooth muscle cells across the internal elastic lamina from the media. Following the migration of smooth muscle cells across the internal elastic lamina, they undergo a rapid proliferation to form a neointima. Endothelial cells migrate from the orifices of branch vessels, forming islands of reendothelialization, which eventually link up (Stemerman *et al.*, 1977). Complete cover by new endothelium may, in the rabbit, take a long time so that at 6 months and even a year following balloon removal of the endothelium there may be areas lacking endothelial cover (Moore and Ihnatowycz, 1978). The delineation of the pattern of endothelial regrowth is greatly facilitated by the intravenous injection of Evans blue dye, which binds to albumin and outlines with clarity the areas that are hyperpermeable to plasma proteins (Stemerman *et al.*, 1977). These correspond on ultrastructural studies to the areas that remain uncovered by endothelium. The areas recovered by endothelium appear white on the blue background of nonreendothelialized areas. Lipid accumulates selectively in the "white" areas as estimated morphologically in oil red-O stained sections and by chemical analysis of pooled intimal–medial tissues. Thus the "blue" areas contain very little or no lipid, which is relatively abundant in the "white" areas (Moore and Ihnatowycz, 1978). The amount of lipid appears to increase with time. The intima of "white" areas is thicker than "blue" areas and abdominal white and blue areas are thicker than thoracic lesions (Moore, 1981).

The mechanisms by which lipid accumulates selectively in the white areas covered by regenerated endothelium may relate in part to an increased production of glycosaminoglycans by the smooth muscle cells of the neointima in these areas. In the blue areas, uncovered by regenerated endothelium, glycosaminoglycan production appears to be much less. Chemical extractions of lipid from blue and white areas (Moore and Nazir, 1979) support the morphological findings in relating the amount of glycosaminoglycan material to the concentration of granules of ruthenium red stained proteoglycans in the interstitium of the neointima (Richardson *et al.*, 1980). The observations suggest that binding of low-density lipoprotein (LDL) to glycosaminoglycans may provide a mechanism for trapping of lipid in the lesions. It has been shown that glycosaminoglycans bind to LDL *in vitro* (Iverius, 1972) and that the complexes so formed can be disassociated by the addition of high-density lipprotein (HDL) (Bihari-Varga, 1978). Such a mechanism would go some way toward explaining the close epidemiological association between low levels of serum HDL and the severity of clinical atherosclerotic disease.

B. The Role of Factors Derived from Platelets in the Response of the Vessel Wall to Injury

1. PLATELET-DERIVED GROWTH FACTOR. From the above account it is likely that platelets, which are prominent in the response of the vessel wall to injury, may have a part to play in the process. The main reaction involves the migration and proliferation of smooth muscle cells. There is now good evidence that platelets that undergo the release reaction release into the surrounding medium a growth factor. This was first clearly shown by Ross and his colleagues in 1974. Since then, a number of workers have essentially confirmed the finding (Pledger *et al.*, 1978). It is apparent also that as well as the growth factor derived from platelets another factor present in serum or plasma is a necessary requirement for smooth muscle cell proliferation to occur (Clemons *et al.*, 1980). We have shown that the platelet-derived growth factor will only stimulate smooth muscle cell proliferation in the presence of serum from platelet-poor plasma, and a dose response to increasing concentrations of serum can be demonstrated (Ihnatowycz *et al.*, 1979a). Similarly, holding the serum concentration constant a similar response can be shown to addition of material derived from increasing numbers of platelets. These findings provide an explanation for the *in vivo* findings of close association of platelets with the vessel wall as an early or initial feature of the response to injury.

Evidence has also come from *in vivo* experiments of two kinds. Induction of a profound thrombocytopenia by the use of antiplatelet serum in rabbits markedly reduced or abolished the development of raised lesions in response to an indwelling aortic catheter (Moore *et al.*, 1976). Similarly, the intimal thickening following balloon removal of the endothelium was markedly inhibited or prevented (Friedman *et al.*, 1977).

Another line of evidence stems from the observation that factor VIII, Von Willebrand factor, has an essential role in the adherence of platelets to the subendothelial connective tissue. Everted segments of rabbit aorta, denuded of endothelium, showed reduction in adhesion of platelets to the surface in an artificial perfusion system when blood from patients with Von Willebrand's disease was employed (Tschopp *et al.*, 1974). Paradoxically, less adhesion occurred at higher rates of shear, which is the reverse of the finding with normal blood. It has recently been shown, employing an antibody to platelet factor 4, that this material rapidly appears in the aortic wall following removal of the endothelium by a balloon catheter (Goldberg *et al.*, 1980). It persists for

some hours and then can no longer be detected. Since platelet-derived growth factor is another protein released from platelets, probably from the α-granules, it may be that it also rapidly enters the vessel wall to stimulate smooth muscle cell replication.

2. PLATELET-DERIVED CHEMOTACTIC FACTOR. The ability of material released from platelets to stimulate the migration of smooth muscle cells has been examined using a modified Boyden chamber. Smooth muscle cells seeded on the top of a millipore filter of an 8.0-μ hole diameter migrate to the lower surface in response to material released from platelets by mechanical disruption, exposure to collagen, or to thrombin. When material released from platelets is also placed in the upper compartment of the chamber no migration occurs. As with platelet-derived growth factor, release of the chemotactic factor is not inhibited by drugs that block the endoperoxide pathway when thrombin or large doses of collagen are used to stimulate platelet release. The platelet-derived chemotactic factor is, however, not dependent on another serum or plasma factor for its activity (Ihnatowycz et *al.*, 1979a, 1981).

All of this evidence supports the concept that the interaction of platelets with the vessel wall stimulates the development of fibrointimal thickenings which may then serve as a substrate for lipid deposition and the development of lesions similar to those seen in human atherosclerosis.

C. Modification of the Response of the Arterial Wall to Injury

The evidence that severe depletion or removal of platelets from the circulating blood inhibits the development of lesions indicates that drugs that interfere with platelet function might modify or ameliorate the development of atherosclerosis. Most interest, in this regard, has been focused on the nonsteroidal antiinflammatory drugs. With some exceptions these have proved disappointing. This may be because, even though some of these drugs can block the conversion of arachidonic acid to prostaglandin precursors and nonprostanoate substances, they do not inhibit the release of other factors from platelets and specifically the platelet-derived growth factor (Ihnatowycz et *al.*, 1979b) and the platelet-derived chemotactic factor (Ihnatowycz et *al.*, 1981) when platelets are exposed to thrombin or large amounts of collagen.

A question remains about the efficacy of dipyridamole. Two experimental studies have shown a lessening of intimal thickening in venoarterial grafts under the influence of aspirin and dipyridamole (Metke *et al.*, 1979; McCann *et al.*, 1980). Dipyridamole has also been shown to inhibit the adherence of platelets to the subendothelial connective tissues (Kinlough-Rathbone *et al.*, (1978). These matters are more fully explored in Chapter 4.

III. THROMBOSIS IN PROGRESSION OF ATHEROSCLEROSIS

One of the least controversial aspects of the relationship of thrombosis to atherosclerosis is the concept that plaques can grow and enlarge by accretion of thrombus. Numerous observations made at postmortem study have supported this mechanism (More and Haust, 1961; Moore, 1976). Moreover, studies of blood flow in artificial systems have provided an explanation for thrombus accretion on the shoulders of projections into the lumen such as that formed by a raised atherosclerotic lesion (Goldsmith and Karino, 1977). The development of vortices allows for the entry of small particles into a territory where they can repeatedly contact each other and the vessel wall. Such flow conditions would favor the deposition of platelets and the development of further thrombus. It also seems likely that at sites where plaques fracture or rupture, thus exposing collagen to the blood stream, thrombus formation will occur.

IV. THROMBOTIC COMPLICATIONS OF ATHEROSCLEROSIS

The clinical events that result from the presence of atherosclerotic disease in arteries are several. They may relate to narrowing of the lumen at sites of severe stenosis, marked dilatation of the vessel due to destruction of the wall with the formation of an aneurysm, or to thrombus formation and its sequelae.

A. Mural Thrombosis and Its Sequelae

1. THROMBOEMBOLISM WITHIN THE CORONARY ARTERIAL SYSTEM. In considering the formation of thrombus in an artery it is important to realize that many of the thrombi that develop are mural rather than occlusive. It is apparent that as platelets aggregate in the early stages of thrombus formation they build up to a critical mass, which then breaks off and is carried distally (Gunning et *al.*, 1964a). This process has been well delineated in man in relation to thromboemboli composed largely of platelets, dislodged from ulcerated plaques at the origin of the internal carotid artery; these temporarily block the retinal circulation causing transient attacks of monocular blindness (Fisher, 1959; Ross-Russel, 1961; Gunning et *al.*, 1964b). A similar mechanism is accepted for the pathogenesis of some attacks of transient cerebral ischemia (Millikan and Siekert, 1955). It seems likely that the same process occurs in the other arterial systems affected by atherosclerosis. Thus, an autopsy showed a statistically significant relationship between complicated atherosclerosis of the aorta proximal to the takeoff of the renal arteries and nephrosclerosis (Moore, 1964). Experiments, where a source of thromboemboli was placed in the thoracic aorta of rabbits (Moore and Mersereau, 1965a,b), dogs, and Macaque monkeys (Moore, 1969), resulted in intimal thickening of the renal cortical arterioles with focal areas of ischemic atrophy of the renal cortex (nephrosclerosis). In rabbits, sustained hypertension developed associated with an initial increase in renal renin (Mersereau et *al.*, 1972) and plasma renin activity (Moore and Gent, 1973). In dogs and monkeys, repeated episodes of embolism were needed to induce sustained hypertension.

2. THE EMBOLIC EFFECTS OF CORONARY THROMBOSIS. Since it is likely that atherosclerotic lesions increase in size and cause progressive narrowing of the lumen by the accretion of thrombus material, episodes of mural thombosis must contribute to the process. While there does not seem to be any consensus about the mechanism by which a thrombus may be initiated in a coronary artery, it seems likely from what we know about the process of thrombosis in arteries that a fracture or disruption of the surface of an atherosclerotic plaque could provide an appropriate stimulus. Both the collagen (Hovig, 1963) so exposed to the blood stream and the atheromatous debris (Lyford et *al.*, 1967) uncovered in the plaque substance could provide a stimulus to platelet aggregation and thrombosis. This mechanism is supported by many

autopsy studies of atherosclerotic coronary arteries (Chandler, 1974). The studies that show this have involved serially sectioning through lesions. The locus or point of disruption can be easily missed in routine sections and even a section a few microns from the lesion may not show the intimal tear.

When a thrombus begins to develop on such a lesion, the usual forces tending to disrupt the fragile mass come into play. In addition, if the lumen is already markedly stenotic the higher velocity of blood through the stenosed segment will increase the tendency to embolism. Recent experimental studies by Uchida and Murao (1974) and Folts *et al.* (1976) indicate that the production of a severe stenosis may also facilitate the development of temporarily occlusive masses of platelet aggregates, presumably by disturbance of flow patterns. While it is possible for any mural thrombus to generate fragments composed of platelets or of platelets and fibrin, it seems likely that the process would be facilitated in a stenotic vessel segment, that is, in the presence of severly stenosing atherosclerosis.

Although this mechanism has been proposed as a cause of sudden cardiac death in man (Mustard *et al.*, 1964; Moore, 1975), it has not found much confirmation in autopsy studies. This may be because it is difficult to find such emboli in the microcirculation in autopsy material (Schwartz and Gerrity, 1975) or because pathologists have not been trained to search for and identify them. There is evidence that, when a diligent search is made, platelet aggregate emboli can be found. Haerem (1972) found them in his study of the hearts of individuals who died suddenly. In a small group of cases of sudden cardiac death, Frink and his colleagues (1978) found nonocclusive mural thrombi in all six hearts and distal microthrombi in four.

In a recent study El-Maraghi and Genton (1980) found platelet-fibrin microthrombi in 13% of cases, more frequently in sudden death cases (20%), and indicated that they were more frequent in a subgroup of patients less than 45 yr of age. Because of the difficulties involved in identifying the process at autopsy it may be more logical to pose the question in animal experiments. A number of experiments provide supporting evidence. Mustard and his colleagues (1964) showed that the injection of adenosine diphosphate into the coronary arteries caused transient platelet aggregates in the myocardial microcirculation that were associated with the development of arrythmias, sudden death, and ischemic damage. In later experiments they explored this further in swine (Jorgensen *et al.*, 1967). Arrythmias, focal small infarcts, larger grossly visible infarcts, and sudden death were all more

numerous when ADP was injected directly into a coronary artery than when it was injected into the left ventricular cavity.

In experiments employing continuous intravenous infusion of norepinephrine in dogs or stress in rats, Haft and his colleagues (1972) demonstrated that platelet microthrombi occurred in the myocardial microcirculation with focal damage to the myocardium. Similarly, Moschos and colleagues (1973) induced thrombosis in the left anterior descending coronary artery of dogs by electric stimulation and showed increased uptake of ^{51}Cr-labeled platelets by the myocardium supplied by this vessel.

Blair *et al.* (1964), employing thrombi experimentally induced in dog coronary arteries by a thrombogenic wire composed of magnesium and aluminum, found that a coiled wire caused an occlusive thrombus whereas a straight wire caused a nonocclusive (mural) thrombus. Paradoxically, mortality in the straight wire group was 35% while in the coiled wire group it was 10%. This is consistent with the concept that mural thrombus might be associated with death by generating platelet-fibrin microemboli which triggered a fatal arrythmia. These authors did not characterize the pattern or extent of myocardial ischemic damage resulting from an occlusive or nonocclusive lesion.

Accordingly, we reexamined the problem using either a straight wire inserted into the artery by open-chest technique or two wires crossed at right angles across the lumen of the vessel (Moore *et al.*, 1981). It was found that when an occlusive thrombus was induced it was always associated with a full thickness infarct. A partial occlusion of the lumen (mural thrombosis) was associated with a variety of focal lesions, presumably of an ischemic nature and resembling lesions that have been described in sudden cardiac death in man in the setting of severe coronary artery disease. Early focal lesions included myofibrillar degeneration (contraction band necrosis), necrosis of fibers, interstitial round cell infiltration, and small infarcts, often of microscopic extent. These lesions were usually located in the inner third of the ventricular wall and were frequently subendocardial. Myofibrillar degeneration, when it occurs in extensive form, has been associated with a temporary interruption of the circulation as in coronary bypass surgery, followed by reflow (Bulkey and Hutchins, 1977). In microscopic form, as seen in the magnesium aluminum wire experiments, it may represent small groups of myocardial fibers temporarily deprived of blood flow by evanescent emboli of platelet aggregates, and in this form resembles lesions found in autopsied cases of sudden cardiac death (Reichenbach and Moss, 1975). The focal round cell infiltrates, similar

to those described by Haerem (1975) in cases of sudden cardiac death in man, may represent a reaction to focal damage to myocardial fibers. The larger areas of myocardial fiber necrosis appearing as microinfarcts or small grossly visible infarcts may represent blockage of the microcirculation by platelet-fibrin emboli.

We have modified this experiment by placing a segment of magnesium–aluminum wire in the left anterior descending coronary artery of dogs by indirect technique employing a Sones' catheter passed by way of a femoral or carotid artery. It was found that a loop on the distal end of the wire was needed for it to lodge in the proximal left anterior descending coronary artery. This provides an experimental setting in which the development of thrombosis and the outcome in terms of occurrence of arrythmias and of death can be studied. In one such study (Moore *et al.*, 1979) the effect of low dose aspirin was compared with high dose aspirin and with no drug received by a control group. There were 10 dogs in each group. All the dogs in the control group died within 48 h. None of the dogs in the low dose aspirin group died. In the high dose aspirin group 3 dogs died within 3 days of wire placement. Significant arrythmias developed earlier in the control group than in the high dose aspirin group and earlier in the high dose group than in the low dose group. In the control group 5 dogs had a completely occlusive thrombus; the other 5 had a mural thrombus. All of the 5 with a mural thrombus had platelet or platelet-fibrin emboli in the territory served by the left anterior descending coronary artery. In 2 of these, which died 15 and 85 min after wire placement, no focal ischemic damage was seen. In the other 3, focal lesions were present in the territory of the LAD. In the high dose aspirin group 4 of the dogs had a completely occlusive lesion associated with a full thickness infarct. Those with a mural thrombus showed more extensive focal ischemic damage than the control group. The low dose aspirin group had no occlusive thrombi and much less focal ischemic damage than the control group.

While these findings are consistent with an effect of aspirin in ameliorating the thrombotic process they pose the problem that part of the effect of aspirin may be antiarrythmic. There is evidence that aspirin inhibits the development of arrythmias following nonthrombotic coronary occlusion (Moschos *et al.*, 1978). However, the absence of occlusive thrombi in the low dose aspirin group and their presence in both the high dose group and the control group suggest a mechanism by which the drug may be influencing the thrombotic process. It is known that the effect of aspirin in blocking the cyclooxygenase, which converts arachidonic acid to prostaglandin precursors leading to the forma-

tion of thromboxane A_2 by the platelets, is a long-lasting one. In contrast, the action on the cyclooxygenase which converts arachidonic acid to prostacyclin in the vessel wall, mainly in the endothelium, is a short-lived effect. In the high dose aspirin group in which repeated doses of aspirin were given daily, the inhibition of prostacyclin production may have facilitated the development of occlusive thrombus. In the low-dose group, given a single dose of aspirin daily (14 or 29 mg/g), prostacyclin production may have been less affected so that there was less tendency for thrombus build up. Similarly, the more extensive focal ischemic damage in the high dose aspirin group may be related to persistence of thromboemboli that had lodged in the microcirculation.

In attempting to relate the occurrence of focal ischemic damage to embolism from mural thrombus ^{111}In-labeled platelets were used. In 10 of 12 dogs there was significantly more radioactivity in the myocardial territory served by the left anterior descending coronary artery which contained a thrombogenic wire than in the posterior wall of the left ventricle or of the right ventricle (Moore *et al.*, 1981).

These experiments indicate that either occlusive or mural thrombosis of a coronary artery may be associated with the occurrence of arrythmias and sudden death. In the experiments with magnesium aluminum wire in the coronary artery, dogs that survived for 3 days did not die later, although in the case of occlusive thrombus there was a very large full thickness infarct involving most of the anterior wall of the left ventricle, the apex, and part of the anterior septum. Deaths that occurred during ECG recording or Holter monitoring were preceded by significant arrythmias—runs of premature ventricular contractions, S-T segment changes or ventricular tachycardia—and were characterized terminally by ventricular fibrillation or ventricular standstill. Thus, death was more closely related to arrythmias than to the character or extent of ischemic myocardial injury. In these experiments arrythmias occurred in the presence of both nonocclusive and occlusive thrombosis of the coronary artery.

If similar mechanisms occur in man in the setting of severe coronary atherosclerosis, a number of clinical observations and pathological correlations are congruent.

With the exclusion of pulmonary embolism, valvular heart disease, or primary myocardial disease, sudden death of presumed cardiac origin is associated with the finding at autopsy of severe narrowing of a coronary artery or of several or all the main coronary arteries (Friedman *et al.*, 1975; Lie and Titus, 1975; Kuller *et al.*, 1975; Rissanen, 1979). There may or may not be an occlusive coronary thrombus, and the incidence of this finding varies widely in different series. Thus, there is

general agreement that the predisposition to sudden cardiac death is severe stenosing coronary atherosclerosis. Many of those who die never reach the hospital. The development of emergency care facilities (Pantridge and Geddes, 1967) has provided the opportunity to study the out come of those who have been resuscitated from ventricular fibrillation. Cobb and his colleagues (1975) in Seattle have reported on the outcome of 234 such patients, followed for 4 yr. Those who did not develop a significant myocardial infarction, as determined by distinct ECG changes or elevation of enzymes following the episode, had a survival experience in which death was three times as frequent as those who did develop infarction. The implication is that the development of a complete occlusion confers some immunity to subsequent fatal arrythmia. Where the vessel remains open and presumably in the same unstable state in terms of development of mural thrombosis the individual is at continuing risk for the occurrence of arrythmia. These findings have been reviewed in an editorial in which it is proposed that dead myocardial tissue may be less likely to generate a fatal arrythmia than tissue that is still at risk for ischemic events (Warren, 1974). The syndrome of acute coronary insufficiency or variant angina is probably relevant in these considerations. Repeat angiograms have shown the subsequent development of complete occlusions in such individuals or acute myocardial infarctions occurred (Madias, 1979; Horie et *al.*, 1978a).

B. Occlusive Thrombosis

Interpretation of the findings in a number of autopsy studies has been construed as meaning that occlusive thrombosis in a coronary artery is secondary to the development of myocardial infarction (Branwood and Montgomery, 1956; Spain and Bradess, 1960; Roberts, 1972; Roberts and Buja, 1972; Baroldi et *al.*, 1974; Silver et *al.*, 1980). There is general agreement that the association of occlusive thrombosis and large (usually full thickness) infarction is close (Chandler et *al.*, 1974; Chandler, 1974, 1975; Horie et *al.*, 1978b). The timing of the finding of a complete occlusion in relation to the beginning of the clinical onset of the heart attack, with some series showing an increasing association of occlusion with time (Spain and Bradess, 1960), has been interpreted to mean that the thrombotic process occurs secondarily in an area of marked stenosis, usually but not always in the vessel supplying the infarcted territory. Although the incidence of arterial thrombi in those dying suddenly varies widely, in some series it has been vey low

(Roberts and Buja, 1972) and has shown an increasing incidence with duration of survival. Those without occlusive thrombus are more likely to have a smaller infarct or subendocardial ischemic damage (Chandler *et al.*, 1974). Thus, the evidence from autopsy studies is conflicting and the originally clear relationship between coronary thrombosis and myocardial infarction, probably first well defined by Herrick (1912) has been brought into question.

The concept that thrombosis occurs as a secondary event is not consistent with what we know about the development of arterial thrombosis. Those supporting the concept have cited evidence that ^{125}I-labeled fibrinogen is taken up by coronary thrombus following the onset of a clinical heart attack (Erhardt *et al.*, 1973, 1976). However, Moschos and colleagues (1976) have shown that an experimentally induced coronary thrombus will continue to take up ^{125}I-labeled fibrinogen for 18 h following its induction. Salimi and colleagues (1977) have reported similar findings. Fulton and Sumner (1977) have shown in clinical studies that the fibrinogen uptake is at the proximal or distal ends of the occluding mass. This is consistent with the development of coagulation thrombus in an area of complete absence of blood flow caused by an occluding platelet-fibrin thrombus.

The concept also loses sight of the fact that thrombosis is a dynamic process in which elements of thrombus accretion and thrombus dissolution are important in the outcome (Mustard, 1976). Examination of the lesion at one time does not predict what may subsequently happen or provide a record of what has happened. A recent report of angiographic findings suggests that the incidence of occlusive thrombosis may decrease with time, showing it to be 87% within 4 h of the onset of symptoms but falling to 65% when patients were studied 12–24 h after onset (De Wood *et al.*, 1980). This is consistent with the concept that thrombosis is an evolving process with factors tending to promote growth of the mass and factors acting to cause its fragmentation or dissolution.

There have been relatively few attempts to explore the problem experimentally, at least in an experimental design that looks at the effects of complete thrombotic occlusion compared to an incomplete thrombotic occlusion. Many of the experiments have used mechanical occlusion, most frequently a ligature applied at the time of open-chest operation or put in place and drawn tight to produce complete occlusion at a later time. In many experiments of this type, animals that survived the arrythmic period did not show, at autopsy, a full thickness infarct. This contrasts with the invariable presence of a full thickness infarct when an experimental occlusive thrombus was induced. This may mean that

there are additional factors augmenting the extent of ischemic injury in a thrombotic occlusion which are not present in mechanical occlusion. An obvious candidate is some material released from aggregating platelets which might influence the process. It is known that thromboxane A_2 is a powerful vasoconstrictor which causes contraction of coronary artery smooth muscle (Ellis *et al.*, 1976). Thus, the release of this material into the coronary circulation may cause constriction of arteries and arterioles accentuating the ischemic damage. It has been shown that the injection of thromboxane A_2 into the coronary arteries causes myocardial infarction (Shimamoto *et al.*, 1977) or focal ischemic necrosis (Morooka *et al.*, 1977). The release of thromboxane A_2 into the local blood supply would also facilitate the aggregation of platelets.

Moschos and colleagues (1973) showed an increased uptake of ^{51}Cr-labeled platelets in the territory served by a coronary artery in which a thrombus had been electrically induced. They attributed this to microcirculatory thrombosis or embolism. Vik-Mo (1978) has shown that following coronary ligation in dogs there was accumulation of platelets in the ischemic and borderline ischemic myocardium 15 min later, which became much more marked at 150 min. In spite of the large amount of platelet trapping at 150 min, the blood flow in the ischemic area increased significantly from 15 to 150 min. He concludes that platelet trapping in the first hours following a nonthrombotic coronary artery occlusion is insufficient to impair coronary circulation.

Somewhat similar experiments have been carried out in nonhuman primates (Leinberger *et al.*, 1979). The ischemic area was defined and mapped by an electrocardiographic prepericardial grid. Occlusion was maintained for 6 h or released at 3 h, reflow being permitted to occur for a further 3 h. All animals were killed 6 h after ligation. Myocardial samples were taken for radioactivity counting from known areas of the grid. The center of the coronary infarct was defined as the territory where S-T segment changes exceeded 4 mV with a loss of more than 50% of the initial RS amplitude and a pathological Q wave. In the reperfusion experiments the highest radioactivity was in the center of the infarct. The border zone showed moderate or no increased activity. After ligation for 6 h (without reperfusion) the center showed considerably less radioactivity but above control tissue values. In the border zone there were areas showing radioactivity five times that of control. The authors conclude that platelet trapping in the marginal zone may be important in infarct expansion, although it is not clear whether the platelet trapping is primary or secondary to ischemic damage at the borders of an evolving (expanding) infarct. In other experiments it was shown that platelet trapping in the marginal zone was reduced from

44% of samples in untreated animals to 25% in those pretreated with aspirin (Ruf *et al.*, 1980). These findings are in conflict with those of Moschos and colleagues (1978), who could not show platelet trapping following mechanical occlusion of a coronary artery in dogs. A possible explanation is the rapid opening up of collateral vessels in the dog as compared to the nonhuman primate. Thus, although the evidence is conflicting, there may be some contribution of platelet aggregation in enlarging the area of infarction following complete occlusion of a coronary artery.

The striking difference in the incidence of myocardial infarction in dogs with a mechanical occlusion as compared to dogs with a thrombotic occlusion is, however, further support for factors related to thrombosis or thromboembolism of the microcirculation which is not overcome by existing marginal or collateral blood supply. One added factor that might be important is spasm of vessels, induced by thromboxane A_2. From the known effects of thromboxane A_2 it is clear that, as well as causing spasm, it causes aggregation of platelets. These effects may be important in the evolution of a mural thrombus to become an occlusive thrombus in a main epicardial coronary artery.

V. CORONARY OCCLUSION, THROMBOEMBOLISM, AND CARDIAC DEATH

The term "coronary occlusion" is sometimes used synonymously with "coronary thrombosis." However, it is clear from a number of careful pathological studies of autopsy material that an occlusive thrombus is not always present (Chandler *et al.*, 1974). Some observers believe that thrombus can accumulate on the luminal surface of a nonruptured atherosclerotic plaque and produce an occlusive lesion (Horn and Finkelstein, 1940; Fisher, 1964; Jorgensen *et al.*, 1971). Many observers using carefully performed serial section studies of coronary arteries have related occlusion to one underlying mechanism—plaque rupture. This was probably first clearly described by Benson in 1926 and has been further delineated in a number of studies since that time (Chandler, 1974; Davies *et al.*, 1979), most convincingly by Constantinides (1966) and by Friedman and Van den Bovenkamp (1966). Our more recent accumulation of knowledge about the factors involved in arterial thrombosis makes it possible to adopt a unitary concept of

plaque rupture as the basis of coronary occlusion developing in the setting of coronary atherosclerosis with plaque formation. However, the actual mechanism of occlusion may differ, depending on whether the plaque rupture is large or small, where it occurs on the plaque, and the depth of the defect. A possible further factor is the amount of damage to or replacement of the underlying media which might influence the capacity of the vessel to constrict in response to thromboxane A_2 and serotonin released from aggregating platelets.

A deep rupture occurring in the proximal part of a long plaque allows blood from the lumen to dissect into the plaque. This will expand the plaque to an extent that it may cause a mechanical occlusion of the vessel which may be rapidly fatal. If a lethal arrythmia does not occur, thrombus may form proximal or distal to the point of occlusion. Alternatively, the flowing blood may dissect off the surface of the plaque which is then carried across the lumen to cause a complete obstruction (Chapman, 1965) with similar outcomes. More superficially placed ruptures and those that occur at the summit of the plaque may, by exposing the collagenous material in the surface of the plaque, lead to rapidly developing thrombosis with embolism of platelet aggregates (Wenger and Bauer, 1958; Haerem, 1971, 1972) or platelet–fibrin masses distally (Mustard, 1974). The thrombus may build up to become completely occlusive. Spasm may be an added factor leading to occlusion. The events described above comprise the lesions described in standard pathological texts as atheromatous occlusion, plaque hemorrhage, and coronary thrombosis. It seems very probable that they are really different outcomes with somewhat differing morphology of one basic process-plaque rupture.

Probably none of these occlusive events is irreversible. If the individual does not succumb to an arrythmia induced by ischemia the occlusion may pass from complete to incomplete (Mitchell and Schwartz, 1963; DeWood *et al.*, 1980). A possible early mechanism is the release of a severe spasm associated with a growing thrombus. Later, recanalization may occur. A number of autopsy studies have shown a decreasing incidence of occlusive lesions as the age of the infarct found at autopsy increases (Mitchell and Schwartz, 1963; Horie *et al.*, 1978b). Some idea of the rapidity with which this may occur can be gained from a recent clinical study of 322 patients admitted within 24 h of infarction (De Wood *et al.*, 1980, previously referred to). These findings are difficult to reconcile with the conclusion, drawn from autopsy studies showing an increasng incidence of coronary thombosis related to time of clinical onset of infarction, that thrombosis is a secondary event. A useful summary of current knowledge of the pa-

thophysiology of myocardial infarction has recently been published (Oliva, 1981).

On balance, the weight of evidence seems to support the classical view that coronary occlusive thrombosis is causally related to large or full thickness myocardial infarct.

A. Subendocardial Infarcts

It seems clear from autopsy studies that small or patchy infarcts often subendocardial in location are much less frequently associated with thrombosis in the epicardial coronary arteries (Friedberg and Horn, 1939; Miller *et al.*, 1951; Chandler *et al.*, 1974). Since there is usually severe stenosis of the arteries (Chandler *et al.*, 1974), it has been suggested that these lesions develop because of inadequate perfuson of the tissue causing a relative ischemia (Friedberg and Horn, 1939). Subendocardial lesions outnumber full thickness (transmural) myocardial infarction in cases of sudden cardiac death (Lie and Titus, 1975; Elliot and Holsinger, 1972). A prior history of unstable angina is often elicited. In the follow-up of patients with subendocardial ischemia the majority experienced angina either stable or unstable, only 24% in one series being free of angina. Twenty-one percent developed transmural infarction in the early follow-up period (Madigan *et al.*, 1976). A possible explanation of these findings is that the cause is embolic from mural nonocclusive thrombus (Mills and Ochsner, 1972). Thus, it is less likely that a thrombus will be detected at autopsy.

B. Intermittent Coronary Occlusion

It is possible that acute coronary insufficiency which is associated with severe stenosis (80–95% stenosis) and reversible S-T segment changes may represent intermittent coronary artery occlusion (Neill *et al.*, 1980). Angiograms repeated 4 months after the initial episode showed complete occlusions in the severely narrowed segments in 9 of 30 patients studied. An experimental analogy to this clinical observation is the occurrence of transient coronary artery occluson when a severe stenosis is produced (Uchida and Murao, 1974; Folts *et al.*, 1976). When a severe stenosis (60–80%) was induced in open-chest dogs, 24 of 35 showed cyclical reductions in flow to near zero with

sudden spontaneous return to near control levels. Aspirin, a drug that inhibits platelet aggregation, abolished the cyclical reductions in flow. Sections of the stenosed segment taken during an episode of occlusion showed the lumen blocked by aggregated platelets (Folts *et al.*, 1976). Prostaglandin I_2 has been reported to eliminate the cyclical reduction in flow (Uchida and Murao, 1979).

1. SPASM. Another mechanism that may account for intermittent coronary artery occlusion is spasm (Hellstrom, 1971, 1979; Chahine and Luchi, 1976; Maseri *et al.*, 1979; Aranda *et al.*, 1980; Turlapaty and Altura, 1980). There has been much speculation on the role of spasm in angina, in sudden cardiac death, and even in myocardial infarction. A number of studies have documented angiographically detected spasm in the course of anginal attacks and as a precursor to the development of myocardial infarction (Oliva and Breckinridge, 1977; Maseri *et al.*, 1978b). Maseri and his coleagues have studied this phenomenon extensively (Maseri *et al.*, 1979). When spasm was observed to occur it had not been preceded by increases in myocardial oxygen consumption (Maseri, 1980). Maseri (1980) believes that stable angina as well as unstable angina may be associated with spasm. Occlusions due to spasm were observed to open up or to persist with the subsequent development of myocardial infarction. Maseri *et al.* (1978a) accord a primary role in these events to spasm, leading to intimal damage, thrombus buildup, hemorrhage, or rupture associated with platelet aggregation and thromboxane A_2 release.

Since Maseri and his colleagues and others have demonstrated by angiography the spontaneous occurrence of spasm in severely stenosed coronary arteries, one must conclude that some severely diseased arteries are capable of spastic contraction. One might, however, question the use of the word spasm to describe narrowing or occluson of vessels, detected as a severe reduction or stoppage of the flow of contrast medium. These changes might just as logically be initiated by the rapid buildup of thrombus associated with plaque rupture or perhaps plaque fissure. The addition of spasm would then be a logical sequel. One of the main difficulties in ascribing a primary role to spasm is to know why the spasm is localized and why it so frequently involves a segment of vessel that is already stenosed. However, there is recent evidence that the occurrence of angiographicaly detected spasm was not associated with a detectable increase in thromboxane B_2 (the end product of thromboxane A_2) in the peripheral blood when low dose aspirin was given to inhibit release of thromboxane A_2 (Chierchia *et al.*, 1980).

Other evidence has also been presented that the appearance of increased levels of thromboxane B_2 in the coronary sinus blood followed rather than preceded the onset of variant angina (Robertson *et al.*, 1980).

However, there may be a problem in attempting to detect release of thromboxane A_2 by measurements of its end product in serum. It has been suggested that thromboxane A_2 may bind covalently to albumin, thus escaping assay as thromboxane B_2 (MacLouf *et al.*, 1980). Other circulating platelet products, β-thromboglobulin and platelet factor-4, have been shown to be elevated within 4 h of attacks of variant angina (Sobel *et al.*, 1981).

Further studies may reveal the actual sequence of events. In the meantime, there is compelling evidence to consider plaque disruption as the primary event in all of the syndromes associated with acute coronary insufficiency, sudden death, and myocardial infarction. Spasm may well play an important part, determined by the local release of thromboxane A_2 or other vasospastic agents possibly unopposed by prostacyclin, production of which may be inhibited or absent in the severely diseased vessel wall (Dembinska-Kiec *et al.*, 1977, 1979, Sinzinger *et al.*, 1979).

C. Instantaneous and Sudden Cardiac Death

As noted above, sudden cardiac deaths are infrequently associated with the presence of an occlusive coronary thrombus. When the interval between clinical onset is shorter, although plaque rupture may be found, thrombosis is not present (Bashe *et al.*, 1975). Thus, Bashe *et al.* (1975) noted that in five cases, where death occurred within 5 min, there was plaque rupture but no thrombosis. Two cases of death within an hour also showed plaque rupture. In two of these seven cases the small vessels of the myocardium showed focal platelet thrombosis. Friedman and colleagues (1973), who made a study of instantaneous and sudden cardiac death, noted that thrombosis was found in the coronary arteries of only 1 of 25 cases. There is another report of absence of thrombus in the epicardial coronary arteries in cases of cardiac death occurring instantaneously or within 15 min of onset (Jorgensen *et al.*, 1971). These findings are compatible with the breakdown and embolization of early platelet thrombus so that the source of emboli may be inapparent or difficult to find at the time of autopsy. They are also

compatible with the behavior of experimentally induced arterial thrombi which have been observed to build to a critical mass and then suddenly embolize with the cycle of growth and dissolution being repeated a number of times (Gunning et *al.*, 1964a).

VI. SUMMARY

Conflicting findings and their interpretation have lead to various concepts about the role of platelets and thrombosis in coronary artery disease. There is now substantial evidence for the key importance of platelets in the development of early atherosclerotic lesions. Progression of atherosclerosis by further deposition of thrombus is not seriously questioned. The relative importance of thrombosis, embolism, plaque disruption, and spasm in cardiac death and myocardial ischemia and infarction is still controversial. However, it is possible to view all of these events as sequelae to plaque fissure or disruption with outcomes depending on the degree of occlusion that occurs, or on the amount of thrombus or thromboembolism that ensues. Better definition or establishment of these pathogenetic mechanisms has clear indications for therapy designed to modify the thrombotic process or control the arrythmias that may result.

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6 Clinical Pathogenesis and Physiological Processes Involved in Myocardial Infarction and Sudden Cardiac Death

SIDNEY GOLDSTEIN

I. INTRODUCTION

The key to successful therapeutic intervention in coronary heart disease lies in the prevention of sudden cardiac death. In the last decade attention has been drawn to the early prehospital mortality of coronary heart disease. The observation that most of the mortality of coronary heart disease occurs outside the hospital has reinforced the need for better understanding of the mechanism of sudden death and has

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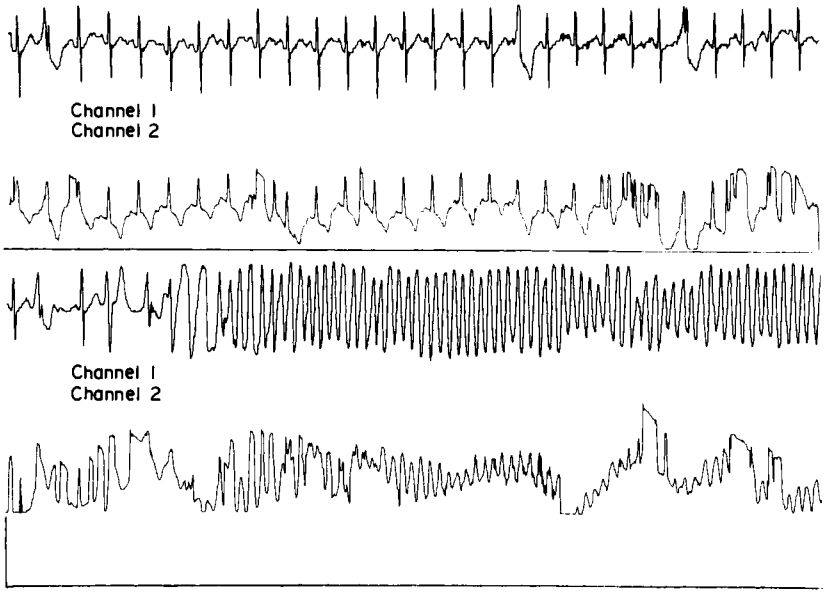

Fig. 1. Ambulatory electrocardiographic recording of the sudden death of a 34-yr-old male occurring 3 months following an acute myocardial infarction. (Reproduced with permission of Dr. David Goldberg.)

emphasized the urgency of interventions directed at the prevention of sudden death. It is clear that any effective therapeutic intervention in coronary heart disease must favorably influence sudden death mortality. Similarly, an intervention that decreases the incidence of sudden death must affect the total coronary heart disease mortality. The sudden death event has been recorded on a number of occasions using ambulatory electrocardiographic recorders. Ventricular fibrillation is the usual event (Fig. 1), although bradyarrhythmias have been observed in 25% of the observed deaths (Iseri *et al.*, 1978). An understanding of the pathophysiologic mechanisms leading up to the arrhythmic event is a requirement for the development of effective therapeutic intervention.

II. THE CLINICAL SETTING OF SUDDEN DEATH

Sudden death due to coronary heart disease represents 76% of all sudden nontraumatic deaths in the adult population. Of all ar-

teriosclerotic heart disease deaths, somewhere between 50 and 80% of individuals die suddenly within 1 h from the onset of symptoms. The time frame in which death occurs has been studied by a number of authors. Bjorck and Wiklin (1972) estimated that over 75% of cardiac deaths within 60 min were witnessed. Kuller *et al.* (1967) observed that 57% of witnessed deaths within 24 h occurred within 1 h of symptoms. Armstrong *et al.* (1972) observed that 45% of all deaths due to coronary heart disease during a 4-week period after the onset of symptoms occurred within the first hour of symptoms. It is also clear that deaths occurring within 1 h are usually instantaneous and often appear to be without symptoms, in the eyes of the witness of the event. In our resuscitated population, however, approximately one-half of the victims recall acute symptoms prior to their sudden death. These symptoms, when present, usually are associated with chest discomfort suggesting acute myocardial ischemia. It is therefore clear that mortality of coronary heart disease is usually sudden, within 1 h and often instantaneous.

Current information suggests that acute transmural infarction or myocardial ischemia are either major precipitating factors or associated with sudden death. Of those individuals resuscitated from sudden death, outside of the hospital, 46% have evidence of either myocardial infarction or necrosis (Baum *et al.*, 1974). Evidence of ischemia or infarction may be present in as many as 73% resuscitated sudden death victims (Liberthson *et al.*, 1974). Our own experience in resuscitated sudden death victims supports the high incidence of infarction and ischemia (Fig. 2). We found (Goldstein *et al.*, 1981) that 78% of these sudden death victims had either evidence of a new transmural infarction (44%) or enzyme evidence of myocardial ischemia (34%) associated with their acute event. Postmortem studies (Baroldi *et al.*, 1979; Rissanen *et al.*, 1978) also emphasize that 77% of sudden death victims have evidence of either infarction or ischemia. Although the major part of the mortality of coronary heart disease is due to acute ischemia and infarctions, a smaller part representing approximately one-fifth of the sudden death victims have evidence of extensive coronary heart disease but appear to die suddenly of primary arrhythmic events. (Gold-

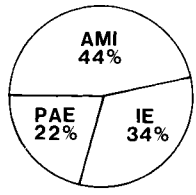

Fig. 2. Classification of the sudden death event was based upon the evaluation of electrocardiographic and enzymatic data in 142 patients with arteriosclerotic heart disease, successfully resuscitated following out-of-hospital sudden death: AMI, acute myocardial infarction; IE, ischemic event; PAE, primary arrhythmic event.

stein *et al.*, 1981) The mechanism of sudden death in this latter group, in whom recent or acute ischemia is not apparent, is more than likely different from the mechanism associated with either acute infarction or myocardial ischemia. It is important to differentiate these two mechanisms in order to develop therapy that is relevant to specific mechanisms. An intervention, for instance, aimed at preventing ischemia could be more effective in preventing ventricular fibrillation occurring as a consequence of ischemia, whereas an antiarrhythmic agent will be more effective in the prevention of primary arrhythmias. In this chapter, we will describe the setting in which sudden death occurs and relate its occurrence to our knowledge of the metabolic and electrophysiologic processes.

III. PATHOLOGY OF SUDDEN DEATH

The extent of cardiac pathology observed in the sudden death victims has provided some insight into pathologic mechanisms. Despite the finding of extensive coronary artery disease in sudden death victims, evidence of acute myocardial infarction is often lacking. Lovegrove and Thompson (1978) reviewed the autopsy findings in 251 cases where death was witnessed, and symptoms preceding death were known to be of less than 30-min duration. Recent myocardial infarction was observed in 20.5% of the patients, and coronary thrombosis was observed in 21.6%. Two-thirds of the sudden deaths occurred without evidence of either myocardial infarction or coronary thrombosis. Evidence of myocardial fibrosis, however, was seen in 74.6% of this population. There were no comments in this study of evidence of early ischemia. Rissanen et *al.* (1978) observed in a detailed postmortem study of 114 cases of witnessed deaths occurring within 24 hr of onset of symptoms that 26% had evidence of a recent transmural myocardial infarction and 51% had evidence of early infarction. Extensive coronary heart disease was observed in all victims. Fifty-six percent had triple-vessel disease, 31% had double-vessel coronary heart disease, and 11% had single-vessel coronary heart disease. Roberts and Jones (1979) studied the extent of coronary atherosclerosis among sudden death victims and found that the process was diffuse, involving all vessels and extending throughout all segments of the vessels. There was no particular propensity for proximal or distal location or localization to one or another of the coronary arteries. The severity of coronary

arterial narrowing could not be correlated with age or sex, angina pectoris, cardiac weight, or the presence of transmural left ventricular scars. Friedman *et al.* (1973) related coronary arterial pathology to the timing of death by dividing their patients into those who died instantaneously (less than 1 min) and those dying suddenly (between 1 min and 1 day). They observed that hearts of the instantaneous group had totally obstructed coronary arteries, particularly of the left anterior coronary artery, more frequently than the sudden death group. They also observed that acute pathology of the coronary vessel was rarely seen in the instantaneous groups but frequently observed in the sudden death group. Silver *et al.* (1980) studied the extent and pathology of coronary arterial disease in 208 witnessed deaths due to cardiac disease. Evidence of acute infarction was present in only 35 of the 208 autopsies. Necrosis, however, resembling that induced by catecholamine infusion, was a unique myocardial lesion observed in 149 of the sudden death victims, 72% in all. This lesion was also present in 29 of 35 subjects with myocardial infarction and found in 85% of the entire group of 208 autopsies. They concluded that there is no relationship between sudden death and the presence of occlusive coronary disease, particularly thrombosis, and that there is no relationship between the degree of coronary artery disease and sudden death. They proposed that a primitive sympathetic disorder may be responsible for ventricular fibrillation leading to myocardial necrosis, fibrosis, and cardiac hypertrophy.

An alternative method of dealing with the pathology of sudden death is the angiographic examination of patients resuscitated outside the hospital from sudden death. It has been observed that 21% of individuals with primary ventricular fibrillation, due to a variety of cardiac diseases who arrest outside the hospital had normal coronary arteriograms and three of them had no evidence of heart disease (Weaver *et al.*, 1976). In those persons who had coronary artery disease (79%), the left anterior descending coronary artery was significantly obstructed in 56%, 61% had circumflex coronary artery obstruction, and 41% had right coronary artery disease. Left ventricular dysfunction was observed in 69%. Our own angiographic observations support these observations. Moderate to severe left ventricular dysfunction was seen in 20 of 33 patients studied after their sudden death resuscitation. (Goldstein, S. Unpublished data)

In terms of the pathology of the sudden death victims, extensive coronary heart disease is present in the vast majority of individuals, often associated with significant left ventricular dysfunction. Although recent myocardial infarction is present at autopsy examinations of ap-

proximately 25% of the sudden death victims, evidence of recent myocardial ischemia is common, often occurring in the setting of preexisting myocardial fibrosis, hypertrophy, and infarction.

IV. THE CLINICAL PRECURSORS OF SUDDEN DEATH

Preexisting cardiovascular disease evidenced by angina pectoris or prior myocardial infarction is usually present in patients prior to their sudden death. Our own study (Goldstein *et al.*, 1981) indicates that 65% of patients dying suddenly had prior myocardial infarction. Kuller *et al.* (1967) indicated that one-half of all arteriosclerotic heart disease sudden death victims between the ages of 40 and 64 had a prior history of heart disease. Cobb *et al.* (1975), in a study of the resuscitated sudden death victim, indicated that 64% of their patients had a history of coronary heart disease prior to their sudden death. Our own studies indicated that the vast majority of sudden deaths outside of the hospital has a history of either myocardial infarction, angina pectoris, hypertension, or congestive heart failure. In the Framingham (Gordon and Kannel, 1971) study, 66% of the patients who died suddenly outside of the hospital had evidence of coronary heart disease prior to their death at the time of a routine follow-up physical examination; and in a study by Fulton *et al.* (1969) in Scotland, it was observed that as many as 65% of sudden deaths had a history of ischemic heart disease. Death certainly, therefore, occurs suddenly but not without warning in the vast majority of instances.

Angina pectoris, clearly, is one of those major antecedants of sudden death, occurring in approximately 50% of all individuals dying suddenly. The mortality rate of angina, of itself, is deceptively benign; however, because of its prevalence, it is a very important factor. The mortality rate of angina pectoris over a 4.5-yr period following the onset of symptoms was 17.5% (Frank *et al.*, 1973). There are, however, undoubtedly subgroups at higher risks. In 140 patients with preinfarction or unstable angina, Gazes was able to identify a subgroup of patients with a mortality rate of 41.4% within 8 months of the diagnosis (Gazes *et al.*, 1973). The association of ventricular premature beats with angina is an added risk factor. Ruberman *et al.* (1980) demonstrated that the mortality rate in patients with complex ventricular premature beats occurring in patients with angina and without a prior myocardial

infarction was twice that of those without ventricular premature beats. The presence of myocardial infarction in the angina group increased the 5-yr mortality rate from 18 to 24.3%. Data regarding the angiographics extent of anatomic disease in patients with angina pectoris have been obtained in a number of different studies and indicate that the more extensive the vascular and ventricular involvement the greater the mortality rate.

In addition to angina pectoris, patients who have experienced a *myocardial infarction* represent a significant population at risk for subsequent death and particularly sudden death. The estimated mortality rate after discharge from the hospital is approximately 10% in the first year and subsequent annual mortality is between 3 and 5%. The mode of death in these patients varies widely but sudden death certainly continues to represent the vast majority of deaths. Pell and D'Alonzo (1964) observed a 5-yr mortality of 26%. Previous studies had indicated a 5-yr survival rate varying from 17 to 51%. They observed that the mortality rate was related both to age and hypertension. Kannel *et al.* (1979) in the Framingham study observed that 1 in 5 men who had a first myocardial infarction died within the first year of the event. This mortality rate includes deaths in the hospital and in the post hospital period. Sixteen percent of the deaths occurred in the first days with an additional 3% occurring during the subsequent year following discharge. Increased mortality rate was associated with development of congestive heart failure. In the study of survivorship following acute myocardial infarction in Sweden it was observed that sudden death was more common in the first year mortality as compared to nonsudden death (Helmers *et al.*, 1976).

A number of studies have attempted to develop risk stratification of patients following myocardial infarction. The strongest association with mortality in the first 6 months, in a study by Bigger *et al.* (1978), was related to the blood urea nitrogen level, serum creatinine level, uric acid level, and cardiac enlargement and ventricular tachycardia after infarction and evidence of ventricular failure during the coronary care unit stay. They observed 15 deaths within the first 6 months in 100 patients; 12 of these deaths were considered to be sudden within 24 h of the onset of symptoms. Davis *et al.* (1979) studied 940 patients who survived hospitalization in Rochester, New York. They observed a 12-month mortality rate of 8% and a 3-yr mortality rate of 12%. A combination of anterior myocardial infarction with ventricular dysfunction and ventricular premature beats identified a high risk subset which made up approximately 15% of the entire myocardial infarction population. This group had a 6-month mortality of 15% and a 3-yr mortality of 30%.

A low risk subset, making up 24% of the postmyocardial infarction population, had a 3-yr mortality of 6%. Premature ventricular complexes were observed to be more frequent in patients with larger infarctions, and the combination of ventricular premature contraction and large infarctions was associated with an increased early and late mortality following acute myocardial infarction (Geltman *et al.*, 1979).

This mortality incidence is not just confined to patients admitted to the CCU with an acute myocardial infarction, but is similar in patients admitted to the CCU with coronary artery disease with chest pain without infarction. In a coronary care unit study, patients in whom myocardial infarction was ruled out and who had coronary heart disease were compared to patients who suffered an acute myocardial infarction (Schroeder *et al.*, 1980). There was no significant difference in the long-term mortality in these two populations. The presence of cardiomegaly, congestive heart failure, and persistent angina after discharge tend to increase the risk of morbidity and mortality in both groups.

The electrocardiogram has long been used as an epidemiologic screening technique and a prognostic indicator in coronary heart disease. In the Tecumseh study (Chiang *et al.*, 1969), it was observed that the presence of a premature ventricular contraction occurring on a routine 12-lead electrocardiogram predicted 38 of 45 sudden deaths that occurred in that population. The landmark studies by Hinkle *et al.* (1974) established a new research direction in which long-term electrocardiographic tape recordings became both a diagnostic and research tool. He studied 301 actively employed American males with a mean age of 55 and observed that a frequency of 10 ventricular premature beats per 1000 complexes characterized a group of individuals who had an increased sudden death mortality. Kotler *et al.* (1973) found that a ventricular premature frequency greater than 10/h on an 8–12-h electrocardiographic ambulatory recording in patients recovering from myocardial infarction was associated with a 13.8% incidence of sudden death over a follow-up period of 3 to 54 months. Ruberman *et al.* (1977) studied the association of sudden death and ventricular premature beats in 1739 men monitored for 1 h during the first year after myocardial infarction. They observed that complex ventricular premature beats, characterized as R on T, repetitive, multiform, and bigeminal, were associated 3-fold risk of sudden death. They also observed that complex ventricular premature beats were an independent variable which, when combined with the presence of congestive heart failure, was associated with a 6-fold increased risk of sudden death. In a subsequent study of a similar population by Ruberman *et al.* (1980) they observed that the presence of complex ventricular premature beats

in the setting of angina pectoris was associated with an increased mortality rate. Their observations indicated that the presence of ventricular premature beats in the setting of angina pectoris with or without a prior myocardial infarction was a predictor of sudden death and total mortality. Increased ventricular premature beats have also been predictive of subsequent mortality in resuscitated sudden death victims, both in our studies (Goldstein *et al.*, 1977) and in those of Cobb *et al.* (1978). The importance of ventricular extrasystoles in patients following acute myocardial infarction was studied by Davis *et al.* (1979). They observed that complex ventricular premature beats were associated with a significant increase in cardiac deaths but did not discriminate between sudden and nonsudden death.

Other electrocardiographic characteristics have been used as predictors of total and sudden death mortality. The Coronary Drug Project (1973), reviewing a number of electrocardiographic parameters, found that S-T segment depression was the most significant electrocardiographic factor in predicting sudden death following acute myocardial infarction. The importance of bundle branch block as an indicator of sudden death was studied in 257 patients with bifascicular and trifascicular conduction defects. Of 50 observed deaths, 27 were sudden, with an overall mortality of 19% at 2 yr and the sudden death mortality of 10% (McAnulty *et al.*, 1978). Sudden death, however, due to bradyarrhythmias was uncommon in patients with bundle branch block.

Thus, a review of the clinical setting in which sudden death occurs indicates that individuals with angina pectoris and prior myocardial infarction, complicated by left ventricular dysfunction and frequent ventricular premature contractions, identifies those individuals who are at increased risk of dying following recovery from an acute myocardial infarction.

V. MECHANISM OF SUDDEN DEATH

The occurrence of myocardial infarction as an important precipitating event has been identified in previous studies. As can be seen in Fig. 2, from our own study of resuscitated sudden death victims, a transmural myocardial infarction was the primary event leading to sudden death in 44% of all individuals dying suddenly. An additional 34% experienced a nontransmural myocardial infarction. The acute ar-

TABLE I
Causes of Lethal Ventricular Arrhythmias

Coronary vasospasm
Rupture of an atheromatous plaque
Thrombus
Platelet agglutination
Increase oxygen demand

rhythmias associated with sudden death are undoubtedly a consequence of the acute transmural or subendocardial ischemic process. The magnitude of the ischemic process does not appear to be related to the development of a fatal arrhythmia.

The genesis of the process may be found in a number of different pathologic mechanisms leading to ischemia (Table I). The ischemic process may be a transient phenomena as seen with coronary arterial spasm, or associated with small platelet thrombi. The transient phenomenon may not only be due to sudden decrease in oxygen supply but may also result from a sudden increase in oxygen demand associated with exercise, rage, or physiological stress. The ischemic episode may also be due to total occlusion of a large or small vessel, with the magnitude of the response determined by the availability of coronary collateral vessels to the jeopardized area of myocardium. Any one or all of these trigger events can lead to the sudden event as pictured in Fig. 1.

VI. METABOLIC AND ELECTROPHYSIOLOGIC MECHANISMS

The best available animal model of ischemic sudden death in man is that of Harris (1950), Harris and Rojas (1943), and Harris *et al.* (1954) performed over 30 yr ago. As described by Harris, after a two-stage occlusion of the anterior descending coronary artery in dogs, arrhythmias usually occur in two distinct phases. There is an early phase that begins almost immediately after coronary ligation and lasts for a few hours. After a period of quiescence, the late arrhythmic phase begins at about 6 to 9 h and lasts for 24 to 48 h. By 48 h following coronary ligation, spontaneous arrhythmias have nearly subsided but the heart is still more prone to fibrillate if provoked by electrical stimulation. Although this bimodal distribution has not been entirely described in man, great similarities exist to the human experiment. Sud-

den instantaneous death can be equated to the early phase of the Harris model. The later phase in man can be observed in the coronary care unit or posthospital phase of myocardial infarction.

With coronary occlusion a rapid decrease in high energy phosphate bonds and ATP content in the ischemic cells occurs. At this time, the active transport of potassium into the myocardial cells is impaired with a resultant intracellular loss of potassium and accumulation of sodium. Associated with this, anaerobic metabolism results in the accumulation of lactate and lactic acid within the cell leading to a profound decrease first in the intercellular pH and later the intracellular pH. With sustained occlusion, this is predominately an all or none reaction in the infarct area, although release of the occlusion within 20 to 60 min indicates that a large degree of metabolic and histologic repair is possible. The border zone between infarct and normal tissue has become of great interest not only because of the spectrum of metabolic dysfunction observed but also because of resultant electrophysiologic heterogeneity of responsiveness to depolarization and repolarization.

The electrophysiologic, metabolic, and histochemical changes in the border zone of infarcted isolated pig hearts have been studied by Janse *et al.* (1979). They observed that, associated with changes in the electrocardiogram characterized by elevation of the S-T segment, there was a fall in creatinine phosphokinase and a rise in lactate. There was a temporary return of electrical activity within 30–50 min at time when creatinine phosphokinase and lactate continue to change. After two hours of ischemia, the cells became electrophysiologically unresponsive in the central infarct zone. In the ischemic border zone, intermediate metabolic levels were observed, with cells with normal glycogen content interdigitating with cells depleted of glycogen. Following reperfusion, cells with nearly normal transmembrane action potentials are in close proximity to unresponsive cells with low resting potentials. These findings suggest that ischemic border is composed of interdigitating normal and ischemic zones sharply demarcated from each other. They failed to observe any significant transmural electrical potential gradient between multiple sites within the myocardium.

The action potentials recorded from the depolarized muscle cells in the ischemic zone shows slow depolarization when activated. This slow response promotes the development of reentrant activation and ventricular fibrillation in the early moments after coronary occlusion. Conduction slows significantly and long intercellular delays may develop between the activated endocardium and epicardium in the border zones. Fragmentation of the electrical impulse occurs within the infarct and may spread into the border zones. This results in disorganized electrical activity in the ischemic zone leading to ventricular

premature depolarization, ventricular tachycardia, and fibrillation. Heterogeneity of this process is such that the conduction delay in the ischemic zone may be markedly prolonged in some cells but only minimally prolonged in other cells. This slowed conduction associated with this electrical heterogeneity of conduction results in reentrant activity. The emergence of the electrical activity into normal cells may reexcite the myocardium in the border zone of the infarction and elicit ventricular arrhythmias (El-Sherif *et al.*, 1977).

Moe *et al.* (1941) in 1941 reported their observations on the initiation of ventricular fibrillation in the animal model. They observed that an electrical impulse occurring at the very earliest period of the relative refractory period is able to cause an arrhythmic center from which several impulses are discharged at an accelerating rate. These successive stimuli result in progressive decrease in refractory period, leading to accelerated response. Combined with a delay in conduction, it furnishes an ideal condition for reentry impulses. This may be self-limited because of exit and entrance block about the ischemic area. On the other hand, it is possible that the electrophysiologic setting for recurrent arrhythmia is always latent in areas of myocardial tissue, particularly in the area of fibrosis and ischemia. Reentrant activity (Table II), therefore, depends on the presence of inhomogeneity of refractoriness, slowed conduction, intermittent conduction block, and change in the pattern of ventricular depolarization.

Although reentry appears to be the dominant electrophysiologic mechanism, automaticity may also occur. The capability of repetitive depolarization has been observed by several workers. The slow potential activity which is dependent upon slow channel calcium repolarization may be related to calcium channel blocking activity of some drugs. Calcium blocking agents such as Verapamil may inhibit or abolish this abnormal automaticity observed in the ischemic myocardium. In the studies by Janse *et al.* (1980) the micro reentry blocks associated with ischemic ventricular fibrillation seemed to originate from normal tissue during repolarization episodes which could lead to intercellular electrical inhomogeneity. Thus inhomogeneity could cause intercellular energy potential sufficient to initiate focal reentry impulses.

TABLE II
Determinants of Reentry

Inhomogeneity of refractoriness
Slowed conduction
Intermittent conduction block
Change in the pattern of ventricular activitation

In man, Greene et *al.* (1978) assessed ventricular electrical instability in patients who had experienced myocardial infarction. Using electrical stimulation to produce reentrant rhythms in order to predict sudden death in 50 patients with refractory symptomatic ventricular tachycardia, they observed that repetitive ventricular response to a single ventricular pacing stimulus was absent in 12 normals but was present in 44 of 50 patients with recurrent ventricular tachycardia. Of 48 survivors of myocardial infarction, 19 had repetitive ventricular responses. During the next 12 months, 79% of these had symptomatic ventricular arrhythmias or died suddenly, as compared with 4 of 29 patients who did not have repetitive ventricular responses. They reasoned that the demonstration of repetitive ventricular response in the postmyocardial infarction period identified patients prone to the development of life-threatening ventricular instability. Subsequent studies, however, have been unable to reproduce this rather interesting observation.

Josephson et *al.* (1979) have recently been able to record from the left ventricular cavity electrical potentials indicative of reentrant activity. Heretofore, these have only been observed in animal models. During these studies they observed spontaneous initiation of ventricular fibrillation that was very similar to the animal studies of Moe and Harris. Ventricular fibrillation usually began as a rapid accelerating ventricular rhythm in which the local electrogram remained discrete. With progressive shortening of the coupling intervals, degeneration of the electrogram occurred at random. They observed fragmentation and disorganization of local ventricular electrograms which appear to be discrete and localized and did not always spread into contiguous areas. Fragmentation of the electrocardiogram or localized fibrillation was observed in focal areas at the same time of maintenance of normal electrograms in other areas. In the setting of electrical stimulation of the ventricle, increasingly shorter coupling interval of premature beats was required in order to produce the disorganization of the electrogram which could then lead to ventricular fibrillation.

VII. CENTRAL AND AUTONOMIC NERVOUS SYSTEMS AND SUDDEN DEATH

In addition to the metabolic and electrophysiologic phenomenon taking place in the ischemic myocardium there are indications that the

autonomic and central nervous systems have important effects upon the development of ventricular fibrillation. The parasympathetic nervous system through its vagal mediation appears to have a significant effect on suppression of malignant dysrhythmias occurring after myocardial ischemia at which time reentry is a prominent factor. The vagus appears to have little effect on late arrhythmias and may actually exacerbate those related to automaticity. The manner in which the vagus nerve actually effects suppression in malignant dysrhythmias is not entirely clear. One possible mechanism is related to its effect on the slow inward calcium currents secondary to inactivation of the fast response mediate by sodium. Corr *et al.* (1974) suggest that the beneficial effect of vagus is confined to its suppression of the sympathetic norepinephine output from postganglionic sympathetic nerves.

The sympathetic nervous system also appears to have an effect on the development of ventricular fibrillation. Myocardial norepinephine stores become depleted in ischemic region following myocardial infarction in animals. This release of catecholamine in areas of ischemia, large or small, may lead to arrhythmias. The pharmacologic attenuation of cardiac sympathetic nervous system using β- and α-adrenergic blocking and catecholamine depleting agents has provided further suggestion of the role of the sympathetic nervous system. Propranolol, a β-adrenergic blocking agent, has been shown to increase the fibrillation threshold in animal models (Bloor *et al.*, 1975). Clinical studies tend to suggest that treatment with β-adrenergic blocking agents can prevent sudden death in individuals after the occurrence of a myocardial infarction (β-Blocker Heart Attack Trial Research Group, 1981; Norwegian Multicenter Study Group, 1981).

The importance of the central nervous system and psychological stress upon myocardial ischemia is still of great interest. Lown *et al.* (1978) were able to precipitate ventricular arrhythmias during psychological stress in patients prone to ventricular fibrillation. Clinical studies relating to psychological stress have provided tantalizing threads linking emotional stress in man and to the subsequent development of coronary heart disease and sudden death. The work of Friedman and Rosenman (1959) defining the type A personality and its relationship to development of coronary heart disease provides stimulus for a wide variety of psychosocial investigations in the field of coronary heart disease. Greene *et al.* (1972), in his study of the psychological background of sudden death victims, observed that the individual dying suddenly was often one who "has been running sad or disappointed." He found them to be in a state of chronic depression with numerous disappointments, both in the family and at home. Engel (1971) gathered

a wide variety of experiences relating to sudden death and psychological stress. He proposes a mechanism characterized by an instability of the sympathetic and parasympathic nervous systems leading to wide swings of both tachycardia and hypertension and bradycardia and hypotension. In a study of the circumstances associated with sudden death, Myers and Dewar (1975) observed that significant degrees of psychological stress occurred in the 30 min before death and were more common in sudden death victims than in individuals who had survived an acute myocardial infarction. Stress stimulus for the sudden death victims included surgical procedures, being attacked by dogs, or altercations over a game. Others experienced traffic accidents, sudden notification of divorce, or loss of a job associated with their sudden death. Rahe *et al.* (1974) have identified the relationship of a wide variety of social changes that often precede cardiovascular events and particularly sudden death.

A number of studies have also been carried out in animals. Lown *et al.* (1978) studied fibrillation threshold in dogs and observed that there was a decrease in fibrillation threshold after hypothalamic stimulation. Observations by Skinner *et al.* (1982) indicate that the Cannon's "cerebral defense system" appears to be located in the frontal cortex. His studies indicate that the pattern of autonomic regulation that arises from the frontal lobe appears to control the vulnerability of the heart to lethal arrhythmias. He proposes that a single cerebral system made up of mesencephalic reticular formation, frontal granular cortex, and thalamic reticular nucleus provides the cerebral controls of cardiac stress related arrhythmias. It regulates sensory information to the cortex through a thalamic gating mechanism and provides control of the autonomic effectors. The pattern of autonomic regulation appears to control the vulnerability of the heart to lethal arrhythmias that follow myocardial ischemia. Surgical intervention in animals of the pathway between frontal lobe and the brain stem and the cardiovascular center will prevent ventricular fibrillation in ischemic heart of stressed animals. He proposes the development of the pharmacologic agents to produce the same effect.

It appears that the central nervous system can and probably does have an important modifying effect, upon the ischemic event itself. It also is possible that, in some instances, the central nervous system may actively cause the ischemic event leading to sudden cardiac arrest.

The cascade of events resulting from myocardial ischemia appears to move inexorably to either sudden death or myocardial infarction. The progression of this metabolic and electrophysiologic event is modified by a host of anatomic and neurohumoral controls that can either accel-

erate or decelerate the process. In lieu of our ability to prevent the atherosclerotic process itself, medical and surgical efforts must be directed at impacting on these modulating mechanisms. At the present time a number of exciting avenues of intervention are available to prevent sudden coronary heart disease death.

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7 Considerations in the Design, Execution, and Interpretation of Clinical Trials in Cardiac Mortality

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Myocardial Infarction and Cardiac Death

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I. INTRODUCTION

It is estimated that there are more than a million people who suffer a myocardial infarction in any one year in the United States. Of those 400,000 who survive to be discharged from a hospital a further 50,000 can be expected to die within a year, primarily from cardiac causes (National Center for Health Statistics, 1980). These figures clearly demonstrate that both primary and secondary prevention of cardiac mortality remain major priorities in medical research.

Interventions that have been attempted can be classified into three broad categories: drug therapy, surgical procedures, and lifestyle modification, including adjustments in diet, smoking, and level of exercise. Lifestyle changes are not readily amenable to rigorous scientific evaluation because of a lack of patient compliance and the difficulty of randomly allocating patients to the respective intervention and control groups. Surgical procedures have been evaluated in patients with angina but generally prior to myocardial infarction. The majority of trials in the prevention of cardiac mortality and morbidity have evaluated drugs as either therapeutic or preventive agents.

Clearly, primary prevention methods are most desirable, but their evaluation involves considerable practical difficulties, one of which is the lack of compliance with experimental maneuvers among essentially symptom-free populations. In addition, there are special problems relating to the low rate of occurrence of important clinical outcomes such as myocardial infarction and death which necessitate that large numbers of patients be studied. The latter problem may be reduced somewhat by identifying segments of the general population who are at a relatively higher risk of coronary artery disease, for example, subjects with elevated blood pressure or cholesterol levels, smokers, and people with hemostatic abnormalities or increased platelet turnover. Although there have been drug trials aimed at primary prevention, such as that with clofibrate (Committee of Principal Investigators, 1978), they have been relatively few.

The preponderance of recent trials have been addressed to evaluating drug interventions in the secondary prevention of myocardial infarction, particularly with drugs that affect platelet function (Anturane Reinfarction Trial Research Group, 1978, 1980, 1982; Anturan Reinfarction Italian Study Group, 1982; Aspirin Myocardial Infarction Study Research Group, 1980; Breddin, 1977; Coronary Drug Project Research Group, 1976; Elwood and Sweetnam, 1979; Elwood *et al.*,

1974; Persantine Aspirin Reinfarction Study Research Group, 1980) and with β-blocking agents (Anderson *et al.*, 1979; Ahlmark and Saetre, 1976; Baber *et al.*, 1980; Barber *et al.*, 1975; β-Blocker Heart Attack Trial Research Group, 1982; Hansteen *et al.*, 1982; Hjalmarson *et al.*, 1981; Julian *et al.*, 1982; Multicentre International Study, 1977; Norwegian Multicenter Study Group, 1981; Reynolds and Whitlock, 1972; Wilcox *et al.*, 1980a,b; Wilhelmsson *et al.*, 1974). Particular attention will therefore be paid to issues in the design, execution, and interpretation of this type of trial; the basic research principles advocated, however, apply to all types of long-term intervention studies.

II. METHODOLOGICAL STANDARDS IN CLINICAL TRIALS

The concept of the randomized, controlled clinical trial was first promoted by Bradford Hill in the late 1940s and has now become established as the basic research strategy for the sound evaluation of therapeutic and prophylactic regimens. Since that time, various components of the methodology have been refined until there is now general acceptance of the broad methodological requirements for good, long-term intervention studies.

In their review of platelet-inhibiting drugs in the prevention of clinical thrombotic disease, Genton *et al.* (1975) presented a series of methodological standards which, at that time, were considered appropriate scientific prerequisites for good trials in that area. Another group (Genton *et al.*, 1977) used a slightly modified set of standards to review the state of the art of antithrombotic therapy in patients with cerebral ischemia.

The methodological standards which are generally accepted today are listed in Table I and will be discussed in turn.

TABLE I
Methodological Standards

1. Objective	6. Random allocation
2. Intervention	7. Double-blind study
3. Population studied	8. Cointervention and contamination
4. Comparison group	9. Outcome events
5. Feasibility	10. Statistical analysis

A. Objective

The primary objective of the study should be the test of a specific hypothesis relating to the possible benefits of some defined intervention. For this objective to be clear and unambiguous, specific details need to be given about the intervention to be evaluated, about the patient population to which the intervention will be applied and to which the findings of the trial would be applicable, and about the assessment of benefit in terms of which outcome events will be expected to be modified and in what time frame. In large-scale intervention trials there are likely to be secondary objectives in addition to the primary objectives relating to subsets of outcome events and/or specific patient subgroups, as well as to possible adverse drug reactions.

B. Intervention

The intervention to be evaluated must be described in sufficient detail so that it could be replicated exactly by another investigator or by a clinician who wanted to implement the results of the clinical trial in practice. With a drug intervention, therefore, it would be necessary to describe who did what to whom and with what formulation and dose; administered where and under what circumstances; with what dose-adjustments and titrations; when it was initiated, how long it was continued, and what the criteria were for deciding that the doses should be increased, tapered or terminated. The intervention should be consistent with current understanding of human structure and function, pharmacology, and clinical practice. The doses, durations, and dose-settings should either conform to current understanding and convention or be justified if they do not.

In the Anturan Reinfarction Italian Study (ARIS) (1982), the intervention being evaluated was the use of sulfinpyrazone, 400 mg twice daily. However, because of sulfinpyrazone's potent uricosuric effect, the dosage was built up during the first week of treatment; it was 200 mg a day on Days 1 and 2, 200 mg twice daily on Days 3 and 4, 200 mg three times daily on Days 5 and 6, and thereafter 200 mg four times daily, in two divided doses. Patients were started on treatment 15–25 days after their qualifying myocardial infarction.

The intervention, if successful, should be accessible and affordable. Clinicians should generally be sufficiently competent to apply it at a

reasonable cost to patients and to the health care system; and patients must be willing and able to tolerate it.

C. Population Studied

Specific clinical criteria, together with criteria for exclusion, should be defined to describe the patients eligible for consideration for the study. These are required for validation of appropriate patient selection relative to the research objectives, for consistency between collaborating centers, and for replication by other investigators and by practitioners. For example, detailed criteria should be laid out to define the qualifying myocardial infarction in terms of specific changes in the electrocardiogram and specific levels of appropriate serum enzymes, as well as the assessment of ischemic chest pain. Exclusion criteria might include associated comorbid conditions (e.g., cardiomegaly); the presence of other life-limiting diseases (e.g., cancer); contraindications to the test treatment (e.g., history of peptic ulcer or requirement to take platelet suppressant therapy for other disorders); and any geographic inaccessibility that might present difficulty in following the patient closely for a long period of time.

One needs a careful balance between increased heterogeneity of the defined patient population, in order to maximize the patient base for generalization and the opportunity to identify particularly responsive subgroups, and a more selected group which may be more consistent with the rationale behind the test intervention. Since the rationale behind the evaluation of platelet suppressant therapy in the post-MI patient is based on the postulated role of platelets in the development of the atherosclerotic plaque (Ross and Glomset, 1976) and in the etiology of myocardial infarction and cardiac death (Haft, 1979), one would need to give careful thought as to when after the qualifying myocardial infarction the intervention should begin and which subsets of patients are unlikely to benefit and hence should not be included.

In multicenter trials there should be a central adjudicating committee to ensure consistency of diagnosis. Each of the collaborating centers should keep a log of all patients admitted to their respective units with a diagnosis of suspected myocardial infarction and should document those patients who were not admitted to the study. This consistency of definition and assessment is necessary to be able to describe the population to which the results of the study would be generalizable.

D. Comparison Group

When assessing the possible benefit of intervention some sort of standard is clearly needed. In studies of the secondary prevention of myocardial infarction it is customary to compare the outcome for a group of patients to whom the intervention has been applied to a control group. In these circumstances, one should ensure that these two groups of patients are similar in all relevant respects, except in the test treatments received. This implies that satisfactory controls should be concurrent in time and place. If the respective comparison groups are studied at different times, the effect of the test treatment can be confounded by general advances in medical knowledge and care and by changing admitting procedures. Indeed, there is good evidence that mortality from ischemic heart disease has declined sharply and consistently for the past 15 yr (Stern, 1979). Similarly, neither the patients admitted nor the care given in different hospitals can be assumed to be equivalent.

The control group should receive the best treatment currently available. Whereas there are some who would claim that anticoagulants have been shown to be of benefit in postmyocardial infarction patients there is no universal acceptance of this; and in the many recent secondary prevention studies in myocardial infarction assessing the possible benefits of platelet suppressant therapy or β-blockers the control groups, without exception, were given placebos that appeared identical to the test treatment.

E. Feasibility

There must be careful consideration of the number of patients to be studied in order for the experimental treatment to be deemed efficacious. A reasonable knowledge of the natural history of the disease is essential, as is some idea of the possible extent of the intervention benefit. This issue of feasibility is discussed in some detail later but it is now well established that because of the low incidence of the two important clinical outcomes in myocardial infarction studies, namely death and recurrent MI, the number of patients required in any one study is in the thousands rather than the hundreds. Because of this requirement for large numbers of patients, multicenter trials are necessary.

F. Random Allocation

The only valid way to allocate patients (i.e., to avoid bias) to their respective treatment groups is according to some prescribed randomized arrangement. Randomization is also a prerequisite for the valid application of statistical tests in the assessment of differences in outcome between the respective treatment groups.

To be appropriate the method of randomization should preferably use a table of random numbers. Allocation according to hospital number or day of week is open to abuse and is therefore unsatisfactory. Systematic allocation of treatment, for example, to alternate patients, is also invalid since the knowledge of which treatment the next patient is to receive could potentially influence the clinician's decision to select a particular patient. Identification of the two comparative, identical-appearing treatments as A and B should also be avoided since breaking the code on any one patient would then "unblind" the whole study.

Perhaps the best arrangement is to have a coordinating center that must be called by the investigator each time a new patient is to be entered into the study. The coordinating center can then inform the investigator which appropriately coded treatment is to be administered to that particular patient, according to some prescribed randomization scheme developed by, and known only to, the coordinating center.

Ideally, one would want to randomize only when it is certain that a particular patient qualified for the study. Problems are created, however, if the intervention is applied before the diagnosis of myocardial infarction is clearly established, since it is well known that some 30% of cases with suspected myocardial infarction are not subsequently confirmed. In this situation, those cases should be acknowledged in the report, but there is no uniform agreement on whether or not such cases should be included in the final analysis. If they are omitted from the primary assessment of efficacy it is a requirement that the final diagnosis of the qualifying MI should have been made without knowledge of which treatment the patient was receiving.

In the metoprolol study (Hjalmarson *et al.*, 1981) patients were entered into the study as soon as possible and only 58% were subsequently confirmed as definite myocardial infarctions. Among these the mortality rate was 13.7% for those receiving placebo compared with only 2.1% in the placebo group wherein myocardial infarction was not established.

Before randomization, the patients should be stratified with respect to some of the important prognostic factors (e.g., hospital, age, previous

myocardial infarction, etc.) in order to maximize the initial comparability of the two treatment groups. Such stratification also facilitates the evaluation of treatment within defined subgroups, which might show that the benefit of the intervention therapy was related to one or more of the identified risk factors. This guards against missing an important, effective treatment in a subgroup of patients that might have been lost in the global assessment of the total group. There are practical limitations to the number of factors on which one can stratify for any given study, but adjustments for additional prognostic factors can be made *post hoc* by applying appropriate statistical methods in the final analysis of the data.

G. Double-Blind Study

An advantage of the use of an identical-appearing placebo treatment in the control group is that the study can be made double-blind wherein neither the attending physician nor the patient knows which treatment is being administered and, as such, the ascertainment and evaluation of outcomes are likely to be made free from bias. This is particularly important when there may be a subjective element in the assessment of important clinical outcomes such as in the classification of cause of death or the establishment of a recurrence of myocardial infarction.

It is sometimes not possible to maintain blindness because of characteristic side effects or some accidental disclosure of laboratory data which clearly identifies the test treatment. Therefore, every effort should be made to have the final assessment made by someone who is unaware the treatment a particular patient is receiving.

H. Cointervention and Contamination

In evaluating the possible benefits of treatment in the defined population, the comparison or control group should have been given the same care and attention—but not the actual treatment—as those patients receiving the treatment under evaluation. In myocardial infarction trials, for example, this would necessitate each group being treated similarly with respect to digitalis, mobilization, β-blockers, etc. In addition, the initial general care should be described and standardized as much as possible since it may vary from one study to another.

As stated earlier, it is necessary for each patient in the control group to be given a therapy that appears identical to the test treatment in order to minimize possible execution and observation biases among the attending health professionals. If the study is not double-blind the patients in the test treatment group may get a more intensive follow-up than the control group and any apparent benefit to this treatment group may simply be as a result of the extra care and attention received rather than the test medication itself. On the other hand, a more intensive follow-up in the experimental treatment group may cause a bias in the other direction since the opportunity to detect important clinical episodes is increased.

There should be some method of assessing the extent to which compliance with the test medications was achieved during the study. Such methods might include pill counts at the periodic follow-up assessments, blood or urine level determinations, and other appropriate laboratory assessments; it should be remembered for example, that aspirin inhibits the secondary wave of the epinephrine platelet aggregation curve and that sulfinpyrazone lowers the serum uric acid.

It is also important to monitor and minimize possible contaminating therapy. For example, it is essential to ensure that none of the patients in a secondary prevention trial of myocardial infarction is taking aspirin on a regular basis. This is particularly problematic, of course, since so many drugs on the market contain acetylsalicylic acid and are readily available without prescription. Where appropriate, patients should be encouraged to use an alternative to aspirin (e.g., acetaminephen) that does not affect platelet function.

I. Outcome Events

There should be clear and specific criteria that are clinically sensible for the outcome events to be used in assessing the efficacy of the intervention treatment. These might include both fatal and nonfatal events together with the use of appropriate objective accurate methods of assessment. When assessing mortality outcomes, total mortality as well as cause-specific mortality should be considered; in the latter instance autopsy assessment is invaluable and should be carried out in as many cases as possible.

Clinical outcomes such as death and reinfarction as well as measures of patient functional status are certainly relevant. Outcomes of questionable value include reductions in cholesterol levels, platelet sur-

vival, and such measures as electrocardiographic and enzyme changes, (except where, in combination, they form a clinically acceptable definition of myocardial infarction); these outcomes show physiological benefits of the drug but it is not established that any of them are necessarily related to clinical benefits to a patient.

A careful record should be made of all alleged adverse drug reactions such as (1) bleeding or gastrointestinal complaints in trials with platelet suppressant therapy or (2) bradycardia and hypotension in studies with β-blockers. At the same time, it is most unlikely that a clinical trial to assess therapeutic benefit will pick up rare but clinically important adverse drug reactions. At the present time, this requires the application of general adverse drug reaction surveillance systems following release of the drug on the market.

J. Statistical Analysis

The statistical methods used in the analysis should be described clearly. In carrying out the analysis, a demonstration of the comparability of the two groups with respect to important prognostic factors is necessary; attention should be paid to complications caused by incomplete follow-up; mortality from all causes as well as that due to the target clinical condition should be reported. The analysis should include the entire group of patients in the study as well as appropriate subgroups. Conclusions regarding efficacy of the test treatment must be restricted to the categories of patients selected for the study.

Most of these long-term trials have included several outcome events of interest. Although it would be reasonable to record separately outcome events such as recurrence of myocardial infarction and death, in assessing the relative efficacy of treatment with respect to recurrence of myocardial infarction, one would obviously have to include death as one of the outcomes. This could possibly be modified to include only death from, for example, myocardial infarction or be restricted to cardiac death, if the cause of death could be determined with acceptable accuracy. However, there is no clinical justification in considering nonfatal myocardial infarction as an outcome event in itself. Among subjects with multiple outcome events, the date of the first relevant event should be used in the life-table analysis.

There are a number of important issues in the analysis of data from these long-term intervention studies, but there are two in particular about which there is considerable debate. The first involves the best

way to handle patients who deviate significantly from the protocol, for example, those who were not eligible in the first case, who were not compliant with the test treatments, or who even withdrew completely from the study before the intended time. This complex problem is discussed in some detail later. The second area of some controversy involves the multiple examinations of the data from the study and the effect this has on the interpretation of the resulting p-values from the statistical tests of significance. Again, this issue will be discussed in more detail later.

III. CONSIDERATIONS FOR THE DESIGN OF FUTURE STUDIES

It is unlikely that any startling new methodologies are likely to be developed or applied in the foreseeable future. However, in looking over the reports from the several recent large intervention trials in the secondary prevention of myocardial infarction, it can be seen that the tactics used have varied considerably from study to study; and it is therefore appropriate to consider what has been learned from these experiences.

As already discussed, there are a number of basic methodological requirements for the design and execution of good clinical trials. Essentially there should be clear, clinically sensible criteria for entry of patients to the study; there should be clear, clinically acceptable criteria for the outcome events of interest; the patients should be allocated at random to the test treatment or control group; there should be a careful and comprehensive follow-up of all patients randomized; there should be an unbiased ascertainment and assessment of all outcome events; and there should be an appropriate statistical analysis. Finally, there should be adequate numbers of patients available and included in the study to enable valid conclusions to be drawn.

If the above requirements had been applied in a clinically sensible and consistent way, one might have expected the numbers of patients entered into the various secondary prevention studies of myocardial infarction to have been very similar. However, an examination of some of the more recent studies, including three with aspirin (Aspirin Myocardial Infarction Study Research Group, 1980; Elwood and Sweetnam, 1979; Persantine Aspirin Reinfarction Study Research Group, 1980), two with sulfinpyrazone (Anturane Reinfarction Trial Research Group,

1980; Anturan Reinfarction Italian Study Group, 1982), and three with β-blocking agents (β-Blocker Heart Attack Trial Research Group, 1982; Hjalmarson *et al.*, 1981; Norwegian Multicenter Study Group, 1981;) shows enormous variation in both the number of patients studied and the mean period of follow-up. These data are shown in Table II.

Perhaps the most striking comparison can be made between AMIS and ARIS; not only did the former study have six times as many patients as the second but the patients were followed for twice as long. Clearly, there is an order of magnitude difference in the size of these two studies. Since the feasibility and cost of such studies is directly related to the number of patients to be studied it should be kept in mind what some of these recent studies have cost. For example, the Aspirin Myocardial Infarction Study (AMIS) cost $17 million, at roughly 1978 dollar values. If the same study were to be initiated today the cost would be almost double.

If designs identical to any of the studies listed in Table II were to be employed in the future, it is reasonable to expect to have to increase the size of the new study by a factor of at least four or five! In all the trials recently completed, the control group received a placebo; in the future, the control group will be receiving one or other of the active agents investigated in recent years (i.e., an antiplatelet agent or a β-blocker). Any proposed new intervention would need to be tested against that. This would require much larger studies to be able to demonstrate clinically important differences in outcomes.

It is essential, therefore, that while maintaining sound scientific principles in the design and execution of future trials, appropriate tactics be applied that will minimize the required sample size.

TABLE II
Recent MI Studies

Study	Number of patients	Average follow-up (months)
Elwood	1682	10
AMIS	4524	38
PARIS	1216	41
ART	1558	16
ARIS	727	19
Timolol	1884	17
Metoprolol	1395	3
Propranolol	3837	25

A. Estimation of Sample Size

The estimated number of patients required in a clinical trial depends upon four parameters: (1) the rate at which the principal outcome events can be expected to occur in the control group (π); (2) the postulated benefit of the test treatment, in effect, reduction in the expected rate at which the active events occur, due to the test treatment (Δ); (3) the risk in concluding the treatment is of benefit when it is not [Type I error (α); the level of statistical significance]; and (4) the risk in concluding the test treatment is of no benefit when it is really of the magnitude specified in (2) above [Type II error (β); 1-β is called the power of the test].

The risks α and β are virtually fixed by convention and are usually chosen to be 0.05 and 0.10, respectively. The required sample size is therefore essentially a function of only two parameters π and Δ; and the greater π and/or Δ the smaller the sample size required.

The importance of π, the rate at which the outcome events are expected to occur in the control group, and its effect on sample size, can be illustrated as follows. If conventional levels of $\alpha = 0.05$ and $\beta = 0.10$ are assumed, and if a true relative reduction in mortality of 50% is postulated for the test treatment, then corresponding to expected mortality rates of 5 and 10%, respectively, in the control group, the required numbers of patients in each treatment group would be 960 and 460, respectively. Hence by doubling the expected mortality rate in the control group it is possible to more than halve the patients required, thereby producing a considerable saving in effort and expense. If the postulated benefit of the test treatment were a reduction by a factor of only 25% rather than 50%, the corresponding expected numbers of patients required in each treatment group would be 4560 and 2180, respectively. Comparing these with the previous estimates, it can be seen that the effect of doubling the expected benefit of the intervention is to reduce the required sample size by a factor of almost five.

B. Experimental Design

Given that fixed levels of α and β are accepted by convention, there is only one way to influence sample size with respect to these two parameters, and that is the choice of the experimental design.

Most of the studies in the secondary prevention of myocardial infarction have involved a single test treatment to be compared against a

placebo control, and in these circumstances patients were invariably allocated randomly in equal proportions to the two comparison groups. This is an optimal arrangement, statistically, in terms of maximizing the opportunity to demonstrate the benefit of treatment, if it really exists. In the Coronary Drug Project (Coronary Drug Project Research Group, 1973) there were five test treatment groups, and in these circumstances the placebo control group, appropriately, received rather more than twice the number of patients allocated to each of the individual test treatment groups.

This contrasts sharply with PARIS (Persantine Aspirin Reinfarction Study Research Group, 1980) in which each of the two active treatment groups, ASA and ASA-plus-dipyridamole, included 810 patients; however the placebo control group included only 406 instead of the optimal number, relative to the two active treatment groups, of 1140. Presumably, the original intention had been to compare ASA-plus-dipyridamole against ASA!

If it is reasonable to assess whether dipyridamole alone is of benefit in the secondary prevention of myocardial infarction, at the same dosage as used in combination with ASA in the PARIS study, then a factorial design would have been the most efficient. Even though there would then be four treatment groups rather than the three in PARIS, the total number of patients required to maintain the same statistical power would actually be fewer than with three groups. The factorial design enables a third question to be addressed.

With such a design, patients would be allocated to one of four treatments: placebo, ASA, dipyridamole, or ASA-plus-dipyridamole. This is a very powerful design and, in these circumstances, very efficient; it enables an assessment of the average effect of ASA, the average effect of dipyridamole, and the possible synergistic or antagonistic interaction between the two drugs. The design has the further advantage that if one of the two drugs proved to be ineffective, it could be considered simply as a placebo, which would mean that all the patients in the study would be essentially on a placebo or the other drug. This design was used by the Canadian Cooperative Study Group (1978) and by Gent *et al.* (1981) in a study of aspirin and sulfinpyrazone in patients with unstable angina.

C. Factors Influencing π and Δ

There are a number of factors over which the investigator has some control that can influence the expected incidence of outcome events in

the control group (i.e., the risk π) as well as the expected reduction in this incidence due to the test treatment (i.e., the response Δ). These involve patient selection and knowledge of the natural history of the disorder, the choice of outcome events on which to base the primary analysis of efficacy, and the extent to which patients and investigators adhere to the protocol and the rules for disqualifying patients and events when there are significant deviations from the protocol.

Some general considerations involved in patient selection have been discussed earlier, and the point was made that the nature of the research question and the rationale behind the specific intervention were the main determinants in selecting the target population. While maintaining this position, however, specific considerations must still be given to risk and response issues. For example, including patients with unrelated but life-limiting diseases would certainly increase the risk of death; but it is also unlikely that the intervention under study would influence some of these deaths, and hence the expected response to the intervention would be decreased. Most investigators would therefore exclude patients with serious comorbidity. Similarly, in a study to evaluate platelet suppressant therapy in the secondary prevention of myocardial infarction the inclusion of a significant number of patients taking ASA regularly for some associated condition could only reduce both the rate at which outcomes are likely to occur and make it more difficult to demonstrate a true benefit of the drug being studied.

It is known that 25% of cases die within 2 h of suffering an acute myocardial infarction, 35% within 1 day, 50% within 1 month, and nearly 60% within 1 yr. Clearly, therefore, one would want to intervene as soon as possible in order to maximize the expected mortality rate in the control group. However, there are competing considerations relating to the expected benefit of the test treatment.

Problems created by entering the patients before the diagnosis has been firmly established were discussed in the section on random allocation. The inclusion of cases who did not have a myocardial infarction can only reduce both the magnitude of the risk and the extent of the response, and hence more genuine MI cases would need to be included to overcome the damping effect of including inappropriate cases.

Even in established cases, however, one needs to consider not only the high mortality rate but the types of death that occur in the different periods following myocardial infarction. It is known that many of the early deaths are mechanical or electrical, and hence most studies to evaluate platelet suppressant drugs have tended to be initiated some months after the qualifying MI. Experience would suggest that this particular issue, which has a very important influence on the required

size of the study, may not have been given sufficiently careful thought. Both AMIS (Aspirin Myocardial Infarction Study Research Group, 1980) and PARIS (Persantine Aspirin Reinfarction Study Research Group, 1980) entered patients 2 to 60 months after their qualifying MI and about 2-yr post-MI on average. In both these studies the 1-year mortality rate in the control group was only 4%. In contrast, Elwood and Sweetnam (1979) admitted more than half their patients into the study within 1 week of their myocardial infarction, and their 12-month mortality rate in the control group was 14%. More importantly, the observed response to ASA was similar in each of the three studies.

Indeed, there is evidence from an earlier ASA study by Elwood et *al.* (1974) that not only were those patients who entered the study earlier, fewer than 6 weeks after their myocardial infarction, at greater risk of dying during the next year but they may even have been more responsive since it was only in this group than an aspirin benefit was observed. The Anturane Reinfarction Trial Research Group (1980) entered patients into the study 25–35 days after their qualifying myocardial infarction and concluded that it was only over the first 6 months of study treatment, when the patients are known to still be particularly high risk, that the benefit of sulfinpyrazone was observed. On the basis of these latter findings the Persantine Aspirin Reinfarction Study Research Group (1980) also looked at a subgroup of patients in their study who had been entered relatively early, and it was among this group that all the benefit of aspirin was observed.

Although there is still much to learn and understand about the complex pathogenetic mechanisms of acute cardiac events and about the detailed clinical course following a myocardial infarction, experience would suggest that a more careful review of these issues could lead to a more appropriate selection of patients and increased efficiencies from the smaller numbers to be studied.

The simplest and, to the patient, the most important outcome event is death, from any cause. However, whereas the risk of any sort of death is obviously greater than the risk of death from some specific cause, the expected benefit of the test treatment is likely to be limited to cardiac deaths. Since the rationale behind the evaluation of platelet suppressant drugs is related to their potential antithrombotic benefits the inclusion of major clinical events such as nonfatal myocardial infarction as one of the outcomes makes sense because their combination with appropriate fatal events would not only be expected to increase the rate of events in the control group but because these would be events likely to be influenced by treatment.

The Anturan Reinfarction Italian Study Group (1982) went a stage

further; they chose as their primary outcome "thromboembolic events" which included fatal and nonfatal myocardial infarction, sudden death, other cardiac deaths, fatal and nonfatal stroke, TIA and pulmonary embolism. By choosing such a wide cluster of relevant events they were able to show that they needed to study fewer than 1000 patients. Of course, such a clustering is clinically less persuasive than, say, cardiac mortality alone; but the judicious selection and grouping of outcomes is a reasonable tactic in the design and execution of studies with limited numbers of patients available for study.

Obviously, the more the patients and/or the investigators deviate from the established protocol the more difficult it will be to demonstrate a truly beneficial intervention. It is essential, therefore, that the collaborating investigators have a clear understanding of the purpose and procedures of the study and that they are committed to the objectives of the study. A coordinating center should be responsible for the prompt review of cases and feedback to the centers; such careful monitoring of the completeness and accuracy of the accumulating data is most important.

The three main problem areas relevant to protocol adherence are patient eligibility, patient compliance with test medications, and completeness of patients follow-up. The number of patients not meeting the inclusion–exclusion criteria must be minimized. In most of the ineligible patients the intervention under evaluation would not be expected to alter the clinical course, and hence their inclusion in the final analysis only makes it more difficult to demonstrate a true intervention benefit. Similarly, patient compliance with the prescribed treatment medication needs to be maximized. Some patients may wish to withdraw from study treatment because of alleged side effects; however, if after careful clinical evaluation it is considered unlikely that the side effects are truly caused by the test medication or if, indeed, the side effects fairly minor, the patient should be encouraged to take as much of the prescribed treatment medication as possible. Taking some of the test medication is always better than taking none of it.

In spite of all the care and attention in the planning and execution of trials there will always be protocol failures. Patients will occasionally be entered into a study and subsequently found not to meet the entry criteria; there will occasionally be interruptions in treatment of some patients, initiated by either the physician or by the patient; and some patients will drop out of the trial for reasons that may or may not be related to their treatment of their clinical condition. Patients may experience some side effects, either real or apparent; they may suffer some intermediate clinical event that prompts their withdrawal from the

study; or they may become too ill to be maintained in the study. These are all clinically relevant reasons and could be very pertinent to the assessment of the intervention under study. Other reasons patients drop out of long-term clinical studies, less relevant to the assessment of the intervention, include simple loss of interest or a move from the immediate geographic area.

D. Intent-To-Treat Versus Efficacy

In the assessment of the intervention under study there is no uniform agreement on how to deal appropriately with protocol deviants. Various positions have been taken (Armitage, 1979; Gent and Sackett, 1979; Peto *et al.*, 1976; Sackett and Gent, 1979; Schwartz and Lellouch, 1967), resulting in a classification split into what have become known as "intent-to-treat" studies and "efficacy" studies.

In an intent-to-treat study once a patient is randomly allocated to one of the treatment groups, any outcome events experienced by that patient during the intended period of study must be counted and charged against the treatment to which the patient was allocated. This is a valid strategy; it is free from bias, and is probably a reasonable representation of clinical care in the real world, hence allowing some measure of the impact of the intention to treat. However, by retaining patients subsequently shown to have been entered in error and eligible patients who did not take adequate amounts of the test treatment, or even withdrew entirely from treatment for reasons unrelated to their clinical condition, it is more difficult to show a true benefit of treatment among patients who take their medication as prescribed.

Some would argue that this latter objective (i.e., assessment of efficacy) is more important than the assessment of the impact of a policy of treatment. In order to maximize the chance of demonstrating the efficacy of a particular intervention one might agree, at the start of the study, on a set of rules under which to exclude events considered unlikely to be influenced by the test treatment.

This strategy was employed by the Anturane Reinfarction Trial Research Group (1978) and the Canadian Cooperative Study Group (1978). A specific tactic employed in both studies was to exclude from the primary analysis of efficacy any patient who did not meet the entry criteria (i.e., who had been simply entered by mistake). To avoid bias, the review of cases and the final decisions on eligibility should be made by a small panel that is unaware of the patient's test treatment or

outcome. The rigorous application of patient eligibility criteria is important since the population to which the findings of the study are generalizable are those patients who have the characteristics defined in the protocol.

A second tactic employed in the same two studies was not to count any events that occurred during the first 7 days of treatment; this was based on the results of a study by Kaegi *et al.* (1975) which suggested that regular administration of sulfinpyrazone in patients with arteriovenous shunts produced clinical evidence of an antithrombotic effect at the end of 1 week. To the extent that this biological premise is correct, a small increase in the sensitivity of the assessment of efficacy should result from not counting events that occurred during the first 7 days.

Kaegi *et al.* (1975) also suggested that the withdrawal of sulfinpyrazone resulted in a loss of antithrombotic benefit within a week; and for this reason the Anturane Reinfarction Trial Research Group (1978) also excluded from their primary analysis of efficacy any deaths that occurred more than 7 days after withdrawal from study treatment unless a subsequent death could clearly be related to some warning event that had taken place while the patient was on study medication. The Canadian Cooperative Study Group (1978) was rather more cautious about the opportunity for bias caused by possible differences between the various treatment groups in their rates of withdrawal of patients related either to the test treatment or to the patient's clinical condition. For this reason they charged any events that occurred within 6 months of complete withdrawal against the corresponding test treatment. This selection of 6 months was arbitrary, but the investigators believed that this tactic was unlikely to show a bias in favor of treatment. The Norwegian Multicenter Study Group (1981) had a similar rule about withdrawal from study treatment, but they chose a 28-day period.

Critics of the above strategy are concerned that the exclusion of any patient after randomization, for whatever reason, creates an opportunity for bias. The proponents, on the other hand, while acknowledging this concern, believe that thoughtful guidelines relating to the conditions under which to count events, together with the establishment of an impartial review process, will avoid any real bias. If bias is, indeed, avoided the expected result of the strategy would be the removal from each treatment group of roughly equal numbers of deaths considered unlikely to be influenced by the study treatments. A comparison of the remaining events provides the most sensitive basis for assessing the efficacy of the intervention.

In the Anturane Reinfarction Trial (Anturane Reinfarction Trial Research Group, 1980) a total of 163 deaths were reported, 89 in the placebo group and 74 in the sulfinpyrazone group. As a result of the strategy described in detail above, 57 deaths were excluded from the primary analysis, 27 from the placebo group and 30 from the sulfinpyrazone group. These numbers are very similar and there is no persuasive evidence of a bias resulting from the strategy.

If these same strategies are applied to the other MI studies that provided the necessary data in their reports, there is no evidence in any of the five major studies of any bias resulting from its application. Indeed, as can be seen from Table III, there was generally a small difference in the other direction.

The Anturane Reinfarction Trial was designed and analyzed as an efficacy study; based on a study sample of 1600 patients an observed reduction in mortality of 30% was found in the sulfinpyrazone group ($p = 0.06$). It can be shown that an intent-to-treat strategy would have required a study sample of 3600 to achieve similar statistical significance. The benefit of the strategy used is clear!

Another tactic that is essentially the same in principle to those described above restricts the analysis to death only from specified causes, for example, instead of considering total mortality one might restrict the assessment of benefit to cardiac death or to sudden death. Surprisingly, critics of strategies similar to those employed in the Anturane Reinfarction Trial are themselves quite willing to employ this last men-

TABLE III
Efficacy Analyses

	Deaths		
	Placebo	Active	A − P
ASA (Elwood and Sweetnam, 1979)	36	27	−9
Sulfinpyrazone (Anturane Reinfarction Trial Research Group, 1980)	27	30	3
Metoprolol (Hjalmarson *et al.*, 1981)	22	19	−3
Timolol (Norwegian Multicenter Study Group, 1981)	35	31	−4
Practolol (Multicentre International Study, 1977)	42	41	−1
Total	162	148	−14

tioned tactic; and yet the expressed concern about the potential for bias applies equally, if not more so, to this situation. Indeed, there are several examples in which the exclusion of certain types of death resulted in a bias that could have led to quite misleading conclusions. One example involves the primary prevention study with clofibrate (Committee of Principal Investigators, 1978) in which there was little difference between the placebo group and the clofibrate group in the number of deaths from ischemic heart disease, but in which there were significantly more deaths from other causes in the clofibrate group!

IV. MULTIPLE ANALYSES

One of the major sources of misunderstanding in the interpretation of the analyses of large-scale intervention studies related to the p values resulting from the application of the various statistical tests of significance. Many people interpret the conclusion that the observed difference in outcome between two treatment groups is statistically significant ($p < 0.05$) to mean that the probability that there is no true difference is less than 1 in 20. This is incorrect.

Even if there is no true benefit of some test treatment compared to the control treatment, the random allocation of patients to the respective treatment groups may by chance alone have put more patients with a good prognosis into one group, and sampling error alone would therefore account for any observed difference in outcome between the two groups. "Statistically significant at the 5% level ($p < 0.05$)" simply means that given the hypothesis that there is no real difference in benefit between the two treatments, the probability of observing as great a difference in outcomes as that shown in the study is less than 1 in 20. Hence, the observed difference is either due to chance, less than 1 in 20, or the hypothesis of no real difference in benefit between the two treatments is untrue. By convention it is inferred from such circumstances that the evidence is sufficiently persuasive to conclude that there is a real difference in benefit between the two treatments.

This reasoning is appropriate if only one question is asked of the data. However, there are often many questions to be examined in these large-scale, long-term intervention studies, and hence there is a very real risk of spurious statistical significance arising from the repeated number of analyses of the data. Suppose, for example, that five statistically independent analyses of the data are carried out; it can be shown

that to maintain an *overall* level of statistical significance of 0.05 the significance level at which each individual test should be carried out is 0.01, that is, 0.05 divided by five.

Since most questions asked are not completely independent, in the statistical sense, the use of the proposed adjustment results in a conservative assessment. For instance, one might be interested in comparing treatment groups on three different outcomes: all deaths, cardiac deaths, and cardiac deaths or nonfatal myocardial infarctions. Since the differences in cardiac deaths will contribute to all three evaluations they are clearly not statistically independent of each other.

To apply the proposed adjustment to the situation in which more than 20 tests are carried out in a particular trial would result in the use of such small p-values that it would be most difficult to demonstrate a valid benefit of treatment. Some investigators (Aspirin Myocardial Infarction Study Research Group, 1980; Persantine Aspirin Reinfarction Study Research Group, 1980) have recommended "caution" in the assessment of statistical significance in these circumstances but were unable to define caution in operational terms. They suggested using a p-value of 0.01, but this is probably less rigorous than it should be in view of the large number of hypotheses tested in each of these two studies.

Multiple examinations of the data from large scale intervention studies can result from (1) the repeated periodic assessment of the accumulating data during the course of the study, (2) there being more than one intervention under investigation, (3) the analysis of different clusters or specific subsets of the various outcome measures, and (4) the evaluation of clinically cogent subgroups of patients.

The justification for the periodic assessment of the data is the ethical requirement that a true superiority of the test treatment, or any significant harmful properties, be identified as quickly as possible. These are not statistically independent examinations, but some adjustment still needs to be made in the final p values to allow for them. With four treatment groups in a factorial arrangement in a study of unstable angina (Gent *et al.*, 1981) there are three principal questions relating to treatment which are essentially independent, statistically. These relate to the overall effect of sulfinpyrazone, the overall effect of aspirin, and the possible synergism between the two study drugs. This must be taken into account when interpreting the p values from tests of specific treatment hypotheses. The most common clustering of outcome events in secondary prevention studies in myocardial infarction is the combination of death, either all or cause-specific, with nonfatal MI; the most common subsets are those for specific causes of death. The An-

turane Reinfarction Trial Research Group (1978, 1980) had cardiac mortality as the outcome event of primary interest, but this was also broken down into three subsets identified as MI deaths, sudden deaths, and other cardiac deaths.

This same group also analyzed these events within specific time periods and concluded that the most striking benefit of treatment appeared to be during the first 6 months of treatment, when these patients are known to be still at particularly high risk, and that the benefit was primarily in terms of an observed 74% reduction in sudden death ($p = 0.003$). This small p value did not impress some of the study critics, even though Tukey, an independent statistical consultant to the study and an international authority on this type of problem (Tukey, 1977), showed that, at worst, the adjustment to be made in the above p value is to multiply by a factor of three to allow for the various combinations of time and type of death. This would result in an adjusted p value of less than 0.01 and hence would still be statistically significant by conventional standards.

Because of the complex pathogenetic mechanisms leading to acute cardiac events it is reasonable to expect that any intervention under assessment may have a significantly greater impact on certain outcomes and within certain patient subgroups. This concept of the identification of highly responsive subgroups of patients has several important implications. It will clearly influence recommendations for patient management; and it may provide some insight into the underlying mechanism of action of platelet suppressant therapy, for example, as well as into the pathogenesis of cardiac disease; and it could influence the design of future intervention studies in this area.

Two approaches to the identification of highly responsive subgroups among patients with transient cerebral ischemia have been discussed by Gent and Hirsh (1979). The first involves looking within a particular study for statistically significant differences in response to therapy between complementary groups; in this way the Canadian Cooperative Study Group (1978) showed a striking difference in response to aspirin between males and females with transient cerebral ischemia. The second approach is to look for consistency of such differences in other studies since this would clearly strengthen the evidence obtained from a single study. Confirmation of a possible sex-related effect of aspirin is provided from both clinical studies (Fields *et al.*, 1977; Harris *et al.*, 1977) and animal studies (Kelton, 1978).

However, a common error in the identification of responsive subgroups from a single study is to show that in a subgroup of patients with a particular clinical or demographic characteristic there is a statis-

tically significant benefit of treatment but that there is not one in those patients without that particular characteristic. The conclusion is then drawn that only those in the former group are truly responsive. Before one can draw conclusions of this nature it must be shown that the *difference* between the two groups in their response to treatment is significantly different from zero. For example, the Canadian Cooperative Study Group (1978) showed that for those patients with a history of hypertension, the observed benefit of aspirin was to reduce the risk of stroke or death by 9%, whereas for those without a history of hypertension the risk reduction was 47%. The former reduction is not statistically significant but the latter is ($p < 0.01$). However, the difference in benefit between the observed risk reductions (i.e., 47 and 9%) is not statistically significant, hence one cannot reject the hypothesis that there is no real difference in response to aspirin by these two complementary groups.

These multiple analyses, among both subsets of outcome events and subgroups of patients, are often referred to as "retrospective analyses." This is generally not the case in practice since they often represent questions identified at the start of the trial. In these circumstances, the only concern is to allow for the multiple tests of significance in the interpretation of the resulting p values. Retrospective analysis is an appropriate description only when it applies to a test that was initiated by an examination of the final outcomes; this is appropriate only for generating hypotheses to be tested in subsequent studies.

V. SUMMARY

Some considerations in the design, execution, and interpretation of clinical trials in cardiac mortality have been reviewed and attention has been drawn to the basic methodological requirements for sound evaluation of new preventive measures. Recognizing the requirement for large numbers of patients in these trials and the expectation that these numbers are likely to be even greater in future trials, particular attention has been focused on various tactics aimed at minimizing these required numbers.

As mentioned earlier, it is known that 25% of cases die within 2 h of an acute myocardial infarction, 50% within 1 month and nearly 60% within 1 year. If one accepts the claim from the Anturane Reinfarction Trial that the study had demonstrated the considerable reduction of

approximately 40% in cardiac death within a year, and allowing for the fact that many patients were excluded from entry to the trial, a reasonable estimate of the saving in lives in the United States alone would be something like 10,000 a year. This is clearly a major advance, but it should be remembered that by the time patients were eligible to enter the Anturane Reinfarction Trial, namely 25–35 days after their qualifying infarction, about half of the MI cases, over 500,000, had already died. This is a sobering statistic and forces a consideration of the need for primary prevention or, alternatively, an intervention that can be applied as soon as possible after the initial heart attack.

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