1971

The Influence Of Beta-adrenergic Receptor Blocking Agents In Experimental Coronary Occlusion In Conscious Dogs

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THE INFLUENCE OF BETA-ADRENERGIC RECEPTOR BLOCKING AGENTS IN EXPERIMENTAL CORONARY OCCLUSION IN CONSCIOUS DOGS

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

FACULTY OF GRADUATE STUDIES
THE UNIVERSITY OF WESTERN ONTARIO
LONDON, CANADA

March, 1971

This investigation was supported by a grant-in-aid from the Ontario Heart Foundation. The author wishes to express his gratitude to this organization.

This thesis is dedicated to

Principal Serajul Islam Khan

ACKNOWLEDGEMENTS

This study was made possible through the generous help and guidance of many people. I should like to thank Dr. G. W. Manning for giving me the opportunity to work in the area of experimental coronary occlusion; it has indeed been a great pleasure to work in the laboratory of a man who has contributed so much in this field. I am grateful to Dr. C. W. Gowdey for encouraging me to enroll in the Ph.D. program as a graduate student in the Department of Pharmacology and for his interest in my work. My sincere thanks are offered to Dr. J. T. Hamilton who volunteered to take over the responsibility of supervising my research when Dr. Gowdey went to England on study leave. The encouragement and advice of Dr. Hamilton have been invaluable during the entire period of this work. His patience in going over my entire work is gratefully appreciated. Dr. M. Roach has been involved in this research from its very inception; her active interest in this project has always been a source of inspiration. Dr. S. P. Ahuja helped me getting started with the experiments and has always been available for consultation. I should like to take this opportunity to thank them all.

Mr. G. C. Stewart has been generous with technical help without which this work would not have been possible. Dr. R. O. Davies of Ayerst Laboratories, Montreal, Canada supplied us with *dl*-propranolol, *d*-propranolol and AY-21,011, and Dr. G. R. McKinney of Mead Johnson Research Centre, Evansville, Indiana provided us with MJ-1999. I should like to thank them for their generous help.

My sincere thanks are extended to Dr. J. Wanklin and Dr. S. Haq for direction and advice regarding statistical analyses, to Mrs. Nancy Smith and Miss Penny Stevenson for surgical assistance, to the Pulmonary Laboratory of Victoria Hospital (Dr. N. Lefcoe, Director) for blood gas analyses, to Mr. B. Bishop, Mr. J. Bryson and Mr. R. Mitchell for preparing histological sections, to Miss Erika Koslowski for invaluable assistance in assembling the bibliography and to Mrs. L. MacLean for typing this thesis.

Finally, I should like to express my gratitude to my parents, who have given me the incentive to seek higher education.

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ABSTRACT

The influence of a group of beta-adrenergic receptor blocking agents has been investigated in experimental coronary occlusion in 141 conscious dogs by tying the most proximal part of the circumflex branch of the left coronary artery. Direct arterial blood pressure and electrocardiogram were monitored for one to two hours or until death. The dogs were randomly allocated:

Group 1: normal saline, 2-3 ml, control, (25 dogs)

Group 2: *dl*-propranolol, 0.1 mg/kg (25 dogs)

Group 3: *dl*-propranolol, 1.0 mg/kg (10 dogs)

Group 4: MJ-1999, 0.2 mg/kg (25 dogs)

Group 5: MJ-1999, 3.2 mg/kg (12 dogs)

Group 6: *d*-propranolol, 0.0723 mg/kg (16 dogs)

Group 7: *d*-propranolol, 0.0723 mg/kg + MJ-1999, 0.2 mg/kg (12 dogs)

Group 8: AY-21,011, 1.4 mg/kg (16 dogs)

The drug or saline was administered intravenously five minutes before coronary occlusion. The doses of these drugs were selected by preliminary experiments whereby their ability to antagonize the chronotropic effect of isoproterenol (1.5 µg/kg) was determined. Thus, the doses of these agents used in Groups 2, 4 and 8 were equiactive and caused 50 percent blockade of isoproterenol-induced tachycardia. The doses used in Groups 3 and 5 caused nearly 100 percent blockade of isoproterenol-induced tachycardia. The dose of *d*-propranolol, used in Groups 6 and 7, has been shown to have equal local anesthetic effect as that of the dose of *dl*-propranolol used in Group 2.

The two-hour mortality rates of 24, 28, 17, 17 and 31 percent in Groups 2, 4, 5, 7 and 8, respectively, were significantly lower than that of 72, 70 and 69 percent in Groups 1, 3 and 6, respectively ($p < 0.001$); all deaths were due to ventricular fibrillation except six dogs which died with cardiac standstill (one in Group 2, three in Group 3 and two in Group 8). Since *dl*-propranolol possesses both beta-blocking and local anesthetic actions and MJ-1999 has only beta-blocking action, it is concluded that the beneficial effect of these agents, in the doses used, is mainly due to their beta receptor blocking property. This is further supported by the lack of similar protection by pre-treatment with *d*-propranolol. Since AY-21,011 is a cardioselective beta-blocking agent and has no local anesthetic effect, it is concluded that blockade of the beta-receptors in the heart alone is sufficient for the beneficial effect.

The higher dose of *dl*-propranolol was associated with an increased incidence of conduction defects, cardiac standstill and higher mortality rate than with the lower dose. This is in contrast with the two dose levels of MJ-1999, both of which reduced mortality after coronary occlusion. For reasons discussed, the dose of AY-21,011 was most likely higher than the ED_{50} doses of *dl*-propranolol and MJ-1999; an increased frequency of conduction defects and cardiac standstill was noted with this dose.

Analysis of arrhythmias revealed that the beta-blocking property of these agents was effective in the protection against ventricular fibrillation following coronary occlusion. Ventricular tachycardia was reduced by both beta-blocking and local anesthetic

actions of appropriate doses of these drugs. Ventricular premature beats, appearing singly or in runs of three or less, were not modified by any of the drugs in doses used in this study.

The relevance of this study to the prophylactic use of beta-adrenergic blocking agents in patients with a risk of sudden death from coronary artery disease is discussed.

INTRODUCTION

Sudden death from coronary artery disease is a major medical challenge. It has been estimated that of over 600,000 patients dying from coronary artery disease each year in the North American continent, approximately 70 percent never reach a hospital (Kuller, Lilienfeld and Fisher, 1966). Death is sudden, unexpected and commonly is nearly instantaneous. There is strong evidence suggesting that the mechanism of sudden death in these patients is ventricular fibrillation (Pantridge and Geddes, 1967; Lawrie, 1969; Lown and Ruberman, 1970).

Sudden death soon after experimental coronary artery occlusion is always due to ventricular fibrillation (Manning, McEachern and Hall, 1939; LeRoy, Fenn and Gilbert, 1942; Harris, Estandia and Tillotson, 1951; Skelton, 1961). General anesthesia reduces the incidence of ventricular fibrillation significantly (Manning, McEachern and Hall, 1939). Investigation into the mechanism of protection by general anesthesia against ventricular fibrillation revealed that sympathectomy in animals reduces the incidence of ventricular fibrillation following coronary occlusion in conscious dogs (McEachern, Manning and Hall, 1940; Skelton, Gergely, Manning and Coles, 1962). Ganglion-blocking drugs have been reported to reduce the incidence of ventricular fibrillation (Regelson, Hoffmeister and Wilkens, 1959; Skelton, 1961). Injection of procaine into the left stellate ganglion has likewise been shown to be beneficial in preventing ventricular fibrillation in anesthetized animals (Milch, Zimdahl, Egan, Hsia, Anderson and David, 1955). In addition, depletion of catecholamines by pre-treatment

with reserpine has been claimed to protect conscious dogs from ventricular fibrillation following coronary occlusion (Hoffmeister, Regelson and Rubin, 1958).

Increased catecholamine concentrations in the urine were found in many patients during the first days after myocardial infarction (Forssman, Hansson and Jensen, 1952; Raab and Gigee, 1954; Valori, Thomas and Shillingford, 1967) and increased levels have been shown to correlate with clinical severity and ventricular arrhythmias (Jewitt, Mercer, Reid, Valori, Thomas and Shillingford, 1969). Plasma catecholamine concentrations following myocardial infarction and after exercise in patients with angina pectoris are higher than those in normal people (Gazes, Richardson and Wood, 1956). The onset of ventricular arrhythmias after coronary occlusion in anesthetized open-chest dogs has been shown to follow the outpouring of adrenaline and/or noradrenaline into the circulation from the adrenal medulla (Staszewska-Barczak and Ceremuzynski, 1968; Ceremuzynski, Staszewska-Barczak and Herbaczynska-Cedro, 1969).

The basic mechanism of 'occlusion fibrillation' is probably intrinsic; but the aforementioned investigations suggest that the sympathetic nerves to the heart and the catecholamines play a very important role in its onset. Since the heart is served by adrenergic beta receptors, beta-adrenergic blocking agents would be expected to protect against ventricular fibrillation and ensuing death soon after coronary occlusion. A search of the literature revealed no study using beta-adrenergic blocking agents in experimental coronary occlusion in conscious animals. Even the number of investigations using anesthetized

open-chest animals is very limited (Pentecost and Austen, 1966; Proger, Sharma and Naimi, 1966; Kauman and Aramendia, 1968; Ceremuzynski *et al.*, 1969). We undertook a study by using *dl*-propranolol in doses of 0.1 mg/kg and 1.0 mg/kg five minutes before coronary occlusion in conscious dogs (Khan and Manning, 1970). These two doses of *dl*-propranolol caused 50 and 100 percent blockade of isoproterenol-induced tachycardia (1.5 µg/kg). The lower dose of *dl*-propranolol was beneficial in preventing occlusion fibrillation and sudden death but the higher dose proved to be cardiodepressive. Racemic propranolol has, in addition to beta-blocking effect, a local anesthetic action (Lucchesi, Whitsitt and Brown, 1966; Barrett and Cullum, 1968). Local anesthetic agents are known to have an anti-arrhythmic effect of their own (Mautz, 1936; Lown, Fakhro, Hood and Thorn, 1967). In order to elucidate as to which of these two major properties of *dl*-propranolol was responsible for the beneficial effect in experimental coronary occlusion, the study was extended to include several other groups of dogs treated with different beta-blocking agents having different pharmacological properties.

This investigation was carried out concurrently in all groups as outlined in the section "Methods and Materials". The research was aimed at finding the answers to a number of questions: whether the beta-adrenergic blocking agents would be beneficial in the prevention of ventricular fibrillation following experimental coronary occlusion in conscious dogs; which pharmacological property of these agents is primarily responsible for the beneficial effect, if any; what effect would the dose level of these drugs have on the final outcome of

coronary occlusion; how would they influence the cardiac arrhythmias immediately following the coronary ligation; what general and cardiovascular effects would these agents have before coronary occlusion and how would they modify the usual response to ligation of a major coronary branch?

This is the first comprehensive report of any such study, using conscious animals, where a number of beta-adrenergic blocking drugs have been used to define the relative roles played by the different properties of these agents on the outcome of coronary occlusion.

HISTORICAL REVIEW

The early history of coronary arteries, the relationship between sudden death and diseased coronary arteries and of experimental coronary artery occlusion has been reviewed by Herrick in his pioneering paper in 1912 and again in a monograph in 1942. (The following pertinent references from the time of Galen to 1912 have been cited from Herrick, 1912, 1942.). The coronary arteries must have been observed long before Galen who is given the credit for coining the term "Coronary". By the sixteenth century, the coronary arteries were portrayed accurately and beautifully by Vesalius (1543) and Leonardo da Vinci (1452-1519). It is during the time of Harvey's *De Motu Cordis* (1628) that the function of the coronary arteries as the nourishing vessels of the heart was clearly understood and even at that time the role played by disease of these arteries was not fully appreciated. Vieussens of Montpellier in 1706 described the course of the coronary arteries. Others referred to "ossification" of these arteries, e.g., Drelincourt (1633-1697), Bellini (1643-1704) and Thebesius (1708). The anastomoses of the coronary arteries were demonstrated by Lower (1631-1691) by experimental injection of one artery from another. Lancisi (1654-1704) of Rome described calcification of coronary arteries as a cause of cardiac enlargement. Senac (1749) described what appears to be thin-walled aneurysm of the left ventricle in association with calcified coronary arteries.

Morgagni (1761) mentioned cases with coronary changes and clinical symptoms like those of angina pectoris or coronary thrombosis. In 1768 and again in 1782 Heberden accurately described and used the

term angina pectoris but he was unaware of any connection of angina pectoris with coronary arteries. However, Fothergill, Parry and Jenner regarded "ossified" and narrowed coronary arteries as the essential lesion in this condition. In 1793 John Hunter, after suffering from angina pectoris for at least 20 years, "dropped down dead" after attending a stormy meeting of the Board of Governors of the St. George Hospital. Autopsy on Hunter was performed by Jenner whose belief in the causal relationship between angina pectoris and coronary artery disease was strengthened by finding severe coronary artery disease in Hunter (Goldstein and Moss, 1969). In 1850 Quain cited 83 cases with clinical features accompanied with both gross and microscopic pathological findings and thus clearly established a relationship.

Obstruction of the coronary artery or of any of its large branches has long been considered to be fatal. Sudden deaths from this cause were described by Lancisi (1654-1704) and Rougnon (1768). Parry, Jenner, Cohnheim (1881) and many others supported this view. Much of the earlier experimental work on lower animals, in which the coronary arteries were obstructed by ligatures, clamps or artificial emboli, gave promptly fatal results. Among those who worked along this line and reached these conclusions are Erichsen (1842), Panum (1862), von Bezold and Samuelson (1880). This uniform, and certain fatal outcome, was thought to be due to lack of anastomosis between the coronary arteries. As late as 1881 Cohnheim, a prominent authority on the subject, lent the influence of his name to the doctrine that the coronary arteries were end-arteries. His necropsy experience and experience on dogs forced him to conclude that sudden occlusion

of one of these vessels meant death within a few minutes. Others emphasized the same view. The subject, therefore, seemed to lack interest from a clinical standpoint. Lack of very obvious auscultatory findings in coronary heart disease has also been responsible for this lack of interest. The unity of coronary artery disease was also obscured by a variety of misleading captions, e.g., angina pectoris, cardiac neurosis, infarct of the myocardium, rupture of the heart, fatty degeneration, acute and chronic myocarditis, acute and chronic heart failure, partial aneurysm of the heart and pericarditis.

But there were dissensions against the Cohnheim doctrine. There were reasons to believe that even large branches of coronary arteries may be occluded without sudden death. By careful dissections, by injection of one artery from another, by skiagraphs of injected arteries and by direct inspection of the hearts made translucent by special methods, there was proof of an anatomic anastomosis that was by no means negligible. Jamin and Merkel's stereoscopic skiagrams (1907), Amenomiya's injection of hearts of young persons showing naked-eye anastomoses in the subepicardial tissue (1910) and Spalteholz's injection and treatment of the heart to make it transparent attest to this (1907). Among others who believed the existence of significant anastomoses between the branches of coronary arteries are Haller, Huehard, Orth, Michaelis, Langer, Legg and West. All recognized, however, that there were individual variations. Michaelis found that the injury from coronary ligation in rabbits was not serious, and was not irreparable in dogs. Renoglio and Drouguell (1888) found that some dogs may survive the insult of coronary ligation. Porter showed

that after ligation of the descending branch of the left coronary artery in dogs, more than half lived. Von Frey, at the Congress of International Medicine in 1891, said that he doubted the sudden stoppage of the heart as Cohnheim taught; he believed that more attention should be given to those where ligation was borne without harm. Hirsch (1907) had no sudden death in eight dogs and two apes. Bickel (1910) had a dog live 19 days after ligation of the anterior descending branch. Kilster (1910) ligated smaller branches; his dogs lived and, when killed at intervals, showed the progressive changes of fibrosis of the myocardium. The more rapidly fatal results obtained by Cohnheim and others were considered to have been due to imperfect technique by which damage was done to the myocardium and pneumothorax produced. Miller and Matthews (1909) obtained better results where ether was used rather that curare and other drugs. With ether-anesthesia, they were able to ligate larger branches with many survivals. Experimentally, then, sudden death was not necessarily the result of obstruction of even large coronary branches.

Herrick (1912) noted that there were numerous autopsy observations, frequently with helpful clinical history, that showed directly or by inference the existence of efficient anastomoses and the ability of the heart at times to survive the obstruction of a coronary artery or some large branch. Thorel (1910) had seen hearts with complete obstruction of the artery, with fibrous or calcified myocardium, and yet no symptom during life, the patient dying of some other disease. Herrick has described a case in whom the descending branch was completely occluded resulting in an extensive fibrous area in the interventricular septum and the apex with an aneurysm at the

latter site. West (1882) cited several cases in which at autopsy complete obstruction of one artery was found, yet patients had long survived the lesion. Merkel (1906) saw in a man the left coronary closed, with nourishment through the right artery. Dock (1896) in a case of gradual occlusion of the right coronary artery was able to demonstrate a direct opening of the finer branches of the left coronary into the end of the right. From these and other observations made by Spalteholz, Recklinghausen, Fujinami, Hirsch, Galli, Samuelson, Osler and Krehl, Herrick emphasized that there was no inherent reason why the occlusion of a large branch of a coronary artery, or even of a main trunk, must, of necessity, cause sudden death. With supporting case reports, Herrick claimed that coronary thrombosis was a definite clinical entity that could be diagnosed during life and was not invariably fatal. He illustrated the variability of the cardiovascular response to coronary occlusion with a wide spectrum of clinical features. He classified the cases of coronary obstruction according to clinical manifestations into four groups (Herrick, 1912, 1919):

1. Cases of instantaneous death.
2. Cases of death within a few minutes or a few hours after the obstruction.
3. Cases of severity in which, however, death is delayed for several hours, days or months, or recovery occurs.
4. A group that was assumed to exist embracing cases with mild symptoms with angina pectoris.

Herrick particularly drew attention to the third group and

claimed that these cases were more common than was generally believed. Experimental work was advanced showing the results of ligation of different branches of coronary arteries. The pathological findings and electrocardiographic changes were compared with human cases and their similarity was demonstrated by Smith (1918) and Herrick (1919).

So far as the experimental coronary occlusion is concerned, Chirac (1698), according to Herrick, was one of the first to venture into this field. The long history of this has led to the elaboration of anatomical, physiological, pathological and clinical knowledge of the coronary circulation. One of the major aspects of modern investigation of diseases of the coronaries has been the study of the mortality rate due to fatal ventricular arrhythmia. An important contribution in this area was made by Manning, McEachern and Hall (1939) who developed a technique whereby a coronary artery could suddenly be occluded in a fully conscious dog. This technique has been adopted by others, e.g., LeRoy, Fenn and Gilbert (1942), Allen and Laadt (1950), Regelson, Hoffmeister and Wilkens (1959) and Skelton (1961). The development of this technique led to a very important observation by Manning *et al.* (1939, 1948). They observed that the mortality rate in dogs after coronary occlusion was significantly lower when the animals were anesthetized. The mortality following sudden occlusion of the anterior descending branch of the left coronary artery in the ether-anesthetized dog was less than 10 percent, for the conscious dog the mortality was 40 percent. For sudden occlusion of the circumflex branch the mortality was 25 percent with anesthesia and 75 percent without. Olichney and Modell (1958) and Hoffmeister, Regelson and Rubin (1958) have confirmed this by demonstrating that anesthesia with pentobarbital

(20-25 mg/kg) and heavy analgesia with morphine sulphate (8-10 mg/kg) also reduced mortality following sudden coronary occlusion.

Sudden death *soon* after experimental coronary occlusion is almost invariably due to ventricular fibrillation. Since anesthesia significantly prevented this fatal arrhythmia, Manning, McEachern and Hall (1939) postulated that the onset of ventricular fibrillation may be due to a reflex mechanism which was depressed by anesthesia. They suggested that sudden accumulation of metabolites in the ischemic area set up afferent sympathetic impulses. These in turn initiated efferent parasympathetic vasoconstrictor impulses which produced spasm of the medium-sized and the smaller coronary arteries and arterioles. This, they suggested, resulted in the production of relatively large areas of ischemia secondary to the primary infarcted area. Under these conditions the inevitably disturbed conduction and contraction led to extrasystoles, tachycardia and fatal fibrillation. If the secondary ischemia were less, they argued, the disturbances would likewise be lessened and fibrillation might not be initiated. Additional support for the reflex coronary artery spasm theory was produced by McEachern, Manning and Hall (1940) who demonstrated that, in conscious dogs bilateral removal of stellate and upper five thoracic ganglia reduced the mortality rate to only nine percent following sudden occlusion of the circumflex branch; the mortality rate from the ligation of the same artery after left-sided unilateral removal of stellate and upper five thoracic ganglia was 33 percent. Sudden occlusion of the anterior descending branch in conscious dogs following bilateral removal of stellate and upper five thoracic ganglia did not cause death in 12 dogs. The

decrease in mortality was suggested to have resulted from the interruption of the reflex arc, preventing the afferent impulses from reaching the vasoconstrictor center and thus eliminating the reflex spasm of the other branches. They, however, considered that the reflex mechanism alone might not be completely responsible for ventricular fibrillation, that sympathetic denervation could have rendered the myocardium less susceptible to the onset of ventricular fibrillation and that both mechanisms might have been involved. That sympathetic denervation increased survival after coronary occlusion has also been shown by others. Even before the study of Manning *et al.*, Leriche, Hermann and Fontaine (1931) had reported that removal of the sympathetic nervous system protected the heart when the anterior descending ramus was ligated in the unconscious dog. Cox, Lewiston and Robertson (1936) reported that the mortality was reduced from 50 percent to 10 percent if the stellate ganglia had been removed before the ligation of this artery. Schauer, Gross and Blum (1937) reported that the incidence of ventricular fibrillation was reduced following coronary occlusion in sympathectomized dogs. The protective effect of sympathectomy was denied by Yodice (1941) who claimed that it was of dubious value if the sudden occlusion was done soon after completion of the denervation procedure and of no value if the occlusion was done 17 days or more after the denervation. Harris, Estandia and Tillotson (1951) agreed that cardiac sympathectomy partially eliminates the excitatory factors which induce ventricular ectopic impulses *soon* after coronary occlusion. Milch, Zimdahl, Egan, Hsia, Anderson and David (1955) also confirmed the beneficial effect of sympathectomy. Subsequently Skelton, Gergely, Manning and Coles (1962) reconfirmed this group's previous study and

further demonstrated that the protection offered by cardiac sympathectomy against fatal ventricular arrhythmia was effective whether the sudden occlusion was done one week or four weeks after surgical cardiac sympathetic denervation.

The concept of reflex coronary artery spasm has not been generally accepted. Eckenhoff, Landmesser and Hafkenschiel (1947) and Wang, Frank, Kenter and Wegria (1957) could not confirm that spasm of non-occluded coronaries followed ligation of one branch. On the other hand, LeRoy, Fenn and Gilbert (1942) have demonstrated photographically that spasm of coronary arteries may occur following ligation of one of its branches. Some support to the "reflex coronary spasm theory" has come from the use of coronary vasodilators by Gold, Travell and Modell (1937), McEachern, Smith and Manning (1941), LeRoy, Fenn and Gilbert (1942) and Gilbert (1942). The results with drugs which cause central depression have not been uniform. Thus Manning and Caudwell (1947) by using demerol and Manning (1948) using morphine found no improvement. On the other hand, Hoffmeister, Regelson and Rubin (1958) found that the mortality was lowered by pre-treatment with each of morphine, pentobarbital and chlorpromazine. Experiments have also been done by interrupting the vagus nerve, the efferent limb of the proposed reflex arc. Manning (1948) found that atropine caused slight improvement in the survival rate following coronary occlusion, but he concluded that the mechanism was uncertain. LeRoy, Fenn and Gilbert (1942), however, strongly endorsed the reflex coronary spasm theory after their study with theobromine, theophylline and atropine. But Essex, Herrick Baldes and Mann (1936) argued that inhibition of vagus results in increased

coronary flow and that the decreased mortality seen after pre-treatment with vagolytic drugs was due to increased coronary flow and not due to interruption of the ischemic reflex.

These differences in opinion are mainly due to two reasons. First, there is a great difficulty in identifying by experimental means the separate effects of nervous influences on the myocardium and the coronary vessels because the physiological functions of these two structures are so intimately related that individual responses may be secondarily modified, each by the other. Second, the fundamental mechanism of ventricular fibrillation is not known. Harris and Rojas (1943) demonstrated that repetitive firing from the ischemic-nonischemic border zone leads to ventricular fibrillation. Harris (1948) noted that dogs subjected to hypoxic hypoxia did not develop cardiac arrhythmia or ventricular fibrillation, the heart simply stopped beating. However, dogs in which a major coronary artery was completely occluded frequently developed ventricular fibrillation. Harris suggested that ventricular fibrillation after coronary occlusion might be explained by the "current of injury" between ischemic and well-perfused areas of myocardium with a concomitant increase in the excitability of the partially ischemic marginal tissue. The absence of ventricular fibrillation in hypoxic dogs was attributed to the absence of adequate current of injury since all parts of the myocardium were more or less uniformly injured. Harris, Bestini, Russel, Brigham and Firestone (1954) produced evidence that ischemic and necrotic muscles release potassium which is the major excitant for the onset of ventricular fibrillation *soon* after coronary occlusion and for ventricular tachycardia which appears 4.5 to 8 hours

later. According to these workers histamine was not an excitatory factor and cardiac sympathomimetic amines were not the sole factor. Additional support came from Cherbakoff, Toyoma and Hamilton (1957) who demonstrated that the incidence of extrasystoles and ventricular fibrillation was reduced if the release of potassium from the ischemic myocardial cells was inhibited by infusion of insulin-glucose, sodium bicarbonate or both. When coronary sinus plasma potassium was low and remained low, ventricular arrhythmias were absent. When coronary sinus plasma potassium was rising, there was a high incidence of arrhythmia and ventricular fibrillation. Brofman, Leighninger and Beck (1956) introduced the concept that the currents between ischemic and well-perfused areas of myocardium were not due to "ischemic injury" but were due to "oxygen differential". They also noted that perfusion of a major coronary artery with oxygenated blood when other portions of the heart were subjected to asphyxia often led to the development of ventricular fibrillation. Badeer and Horvath (1959) argued that according to the theory of "oxygen differential", one would expect that the interruption of the coronary arterial flow to the *entire* heart would not induce ventricular fibrillation. As a matter of fact, it caused ventricular fibrillation in nine out of 10 experiments. Danese (1962) reported that perfusing the left circumflex coronary artery with non-oxygenated homologous serum within 15 to 30 seconds of occlusion, prevented ventricular fibrillation in all of 10 dogs studied, whereas occlusion of the same artery in 30 control dogs led to ventricular fibrillation in 60 percent. Hence, oxygen differential *per se* could not be the responsible factor for the onset of ventricular fibrillation after coronary occlusion. Badeer (1963)

reviewed this subject and thought that the mechanism of ventricular fibrillation is possibly intrinsic, be it due to the absence of substances brought by the plasma (except perhaps oxygen) and/or accumulation of metabolic products. This is possibly modified by extrinsic nerves and hormones. Intracellular microelectrode study has shed some light on this problem. Kardesch, Hogancamp and Bing (1958) have shown that acute ischemia of the myocardium leads to a reduction of the resting membrane potential as well as a reduction in the amplitude and duration of action potential. The reduction of resting membrane potential may lead to an increased rate of firing of the automatic cells. This would also be the result of an increase in the slope of spontaneous phase four depolarization of automatic cells produced by catecholamines. High catecholamine concentrations may so enhance automaticity that multiple pacemakers develop. This can lead to disorganization of activity and fibrillation (Hoffman and Singer, 1967). Shortening of the refractory period may allow hypoxic cells to be re-excited by adjacent normal tissue and lead to fibrillation (Jacobson, Schiess and Moe, 1962).

Harris, Estandia and Tillotson (1951), in an attempt to identify the excitatory factors in the production of ectopic rhythm, described three phases following abrupt coronary occlusion. The first phase was the period of danger of ventricular fibrillation and usually lasted for 10 minutes from the time of occlusion. The ectopic discharges usually began within two to five minutes after occlusion, increased in frequency and precipitated ventricular fibrillation or passed through the maximum frequency and into a decline. The second period began with a decline of the ectopic activity of the first phase and

lasted until the development of ventricular tachycardia of the third phase. The duration of this phase was between 4.5 and 8 hours. The frequency of ectopic beats was very low during this phase. The third phase began 4.5 to 8 hours after the occlusion with a rapid increase in ectopic ventricular beats progressing to ventricular tachycardia. This phase lasted for two to four days and, rarely, five days. Some of the confusion regarding the arrhythmias following sudden coronary occlusion has been due to failure to distinguish between the "early" and "delayed" ventricular arrhythmias.

Whatever may be the basic cause of ventricular fibrillation following sudden coronary occlusion, the sympathetic nerves to the heart and the catecholamines play a very important role in its genesis. The beneficial effect of sympathectomy in the prevention of ventricular fibrillation *soon* after coronary occlusion observed by the many investigators referred to above attest to this. Further support came from the studies where various drugs were used to block the sympathetic outflow. Thus Hoffmeister *et al.* (1958) demonstrated that reserpine, which is known to deplete catecholamines from storage sites, reduced one-hour mortality to 20 percent compared with 66 percent mortality in control dogs following sudden occlusion of the anterior descending artery in the conscious state. Skelton (1961) demonstrated that high epidural anesthesia with lidocaine reduced one-hour and 24-hour mortality compared with control dogs following coronary occlusion. Ganglion-blocking drugs also reduced fatal outcome. Skelton (1961), by using pentolinium bitartrate, and Regelson *et al.* (1959), by using hexamethonium in conscious dogs, have shown this. Injection of

procaine into the left stellate ganglion also protected dogs from ventricular fibrillation and death following coronary occlusion (Milch *et al.*, 1955). Manning and Caudwell (1947) noted that the "sympathetic inhibiting agents", ergotamine and dihydroergotamine also prevented ventricular fibrillation immediately after the occlusion of the circumflex artery. Iproniazid, a monoamineoxidase-inhibitor, was also shown to have a protective effect against sudden death following coronary occlusion (Regelson *et al.*, 1959).

There are other studies which associate catecholamines with postcoronary cardiac arrhythmias. Adrenaline has been shown to be capable of inducing ventricular arrhythmias after acute coronary ligation in dogs by Clark and Cummings (1957). Maling and Moran (1957) demonstrated that adrenaline and noradrenaline were equally potent in inducing ventricular arrhythmia following coronary occlusion and that this exaggerated ectopic ventricular response persisted for about 12 days following the occlusion.

Increased catecholamine concentrations in urine were noted in 14 out of 15 patients with myocardial infarction by Forssman, Hansson and Jensen (1952), in one out of five patients by Raab and Gigee (1954), and in two out of four patients by Nazum and Bischoff (1953). Serial measurements of free adrenaline and noradrenaline in 24-hour urines of four patients with acute myocardial infarction were carried out by Valori, Thomas and Shillingford (1967). The level of excretion of noradrenaline varied from normal values to 800 percent of mean normal level, and of adrenaline from normal values to 350 percent of the mean normal level. There was a tendency towards

a positive correlation between the clinical severity and the urinary level of these catecholamines.

Serial determinations of free noradrenaline and adrenaline excretion in 55 patients with acute myocardial infarction were recorded by Jewitt, Mercer, Reid, Valori, Thomas and Shillingford (1969). In uncomplicated cases, a moderate rise in noradrenaline with only a transient rise in adrenaline was noted in the first week. In contrast, the urinary level of both catecholamines rose notably in patients developing heart failure or cardiogenic shock. In patients with ventricular arrhythmias and multiple forms of arrhythmias in the same patient and in those with ventricular arrhythmia appearing after the third day, the level of both catecholamines was very high. The mean peak excretion of both noradrenaline and adrenaline was significantly higher in nine patients who died than in 46 who survived.

Plasma catecholamine concentrations in acute myocardial infarction and angina pectoris were measured by Gazes, Richardson and Woods (1956). The determinations were made in 13 patients with myocardial infarction within the first 36 hours. Noradrenaline levels were significantly higher than those in normal in all cases. but the adrenaline levels were high in seven cases. There was a significant linear relationship between the plasma levels of noradrenaline and transaminase. Eight out of 12 patients with angina pectoris showed a significant increase in plasma noradrenaline level with exercise, in five of these patients there was also an increase in adrenaline level. Seven normal subjects showed no significant increase in either of these amines following exercise.

The most convincing evidence for the relationship between cardiac rhythm disturbances and the release of catecholamines after acute coronary occlusion has been forwarded by Staszewska-Barczak and Ceremuzynski (1968) and by Ceremuzynski, Staszewska-Barczak and Herbaczynska-Cedro (1969). These studies were made possible by the advent of a sensitive continuous bioassay method to study the release of adrenaline and noradrenaline into the blood stream of dogs before, during and following coronary occlusion (Vane, 1964). Ceremuzynski and associates demonstrated, in anesthetized open-chest dogs, that an increased output of catecholamines into the blood stream, starting within two to 25 minutes of coronary occlusion, occurred in 66 percent of the dogs. Cardiac arrhythmias in the form of unifocal and multifocal ventricular ectopic beats, ventricular tachycardia and ventricular fibrillation developed in over 90 percent of the dogs showing an increased output of catecholamines and in none of the dogs in which an increase in the output of the catecholamines did not occur. Adrenaline was secreted alone or in combination with noradrenaline in most of the cases. The rise of blood adrenaline as a rule preceded by about five minutes the development of the arrhythmias. The output of catecholamines remained high throughout the period of observation of three to four hours. Bilateral adrenalectomy caused disappearance of both adrenaline and noradrenaline, showing that they both were derived from adrenal medulla. The dogs which failed to secrete detectable amounts of catecholamines and showed no ectopic activity after coronary ligation were given infusions of adrenaline and/or noradrenaline intravenously. The rates of infusions were similar to the rates of

spontaneous catecholamine secretion induced by acute coronary occlusion. These infusions given before coronary occlusion produced no disturbances of cardiac rhythm. After coronary ligation, however, in 10 of the 16 dogs, adrenaline provoked cardiac arrhythmia. Noradrenaline infused at similar rates was less active in evoking arrhythmias.

In summary, experimental coronary occlusion frequently leads to ventricular fibrillation. The exact mechanism of this occlusion fibrillation is not quite clear. Sympathetic nerves to the heart and the catecholamines play a significant role in the genesis of this fatal arrhythmia. Sympathectomy, ganglion-blocking agents, epidural anesthesia and catecholamine-depletors have been shown to prevent ventricular fibrillation and death following sudden occlusion of the coronary artery in a significant number of cases.

An entirely new group of drugs having important autonomic effects have been introduced since 1958. These are the beta-adrenergic receptor blocking agents. The history of this group of drugs goes back to 1905 when Langley introduced the concept that effector cells contain excitatory and inhibitory "receptor substances". He thought that the effects of adrenaline are dependent upon the types of receptor substances present in the effector cells (Langley, 1905). This hypothesis was supported by Dale's studies on the adrenergic blocking activity of ergot derivatives (Dale, 1906). Although these drugs completely antagonized and even reversed the excitatory actions of adrenaline, they had no effect on the inhibitory actions. The only excitatory action of adrenaline that was

not inhibited by ordinary doses of ergot was its stimulation of the heart. These results suggested that the effect of adrenaline is dependent on at least two types of receptors, one of which was blocked by ergot. Ahlquist (1948) clearly defined the concept of "alpha"-and "beta"-adrenergic receptors. He studied the relative potencies of six different sympathomimetic amines on a variety of effector systems. When the compounds were tested for their abilities to cause vasoconstriction, excitation of the uterus, contraction of nictitating membrane, dilatation of the pupil and inhibition of the gut, a consistent order of potency emerged. However, this order of potency was found to be altered when the drugs were tested for their ability to produce vasodilatation, inhibition of the uterus, and myocardial stimulation. On the basis of these findings, Ahlquist postulated that two distinct types of adrenergic receptors exist and that the effects of the sympathomimetic drugs on effector cell depend on the type of receptor with which it is capable of reacting. Receptors that, when stimulated, have an excitatory effect were classified as alpha-adrenergic receptors, and those with an inhibitory effect on stimulation were classified as beta-adrenergic receptors. Two exceptions were noted: the cardiac stimulatory effects produced by sympathomimetic amines were noted to be mediated entirely by beta-adrenergic receptors, and the inhibitory effects of sympathomimetic amines on the gut were shown to be mediated by both alpha and beta receptors (Ahlquist and Levy, 1959).

Although a number of alpha-adrenergic receptor blocking agents, e.g., ergot alkaloids, phenoxybenazime and dibenamine, have been known

for some time, it was not until 1958 that Powell and Slater first described the beta-adrenergic receptor blocking property of dichloro analog of isoproterenol (Powell and Slater, 1958). They demonstrated that dichloroisoproterenol blocked the depressor effect on blood pressure of adrenaline and isoproterenol, the uterine relaxation by adrenaline and isoproterenol, increase in blood flow to the hind leg of the dog by intra-arterial injection of isoproterenol, the inhibitory effect of isoproterenol on isolated rabbit intestinal strip, and the effect of isoproterenol or adrenaline on 'ergotoxin-pilocarpine bronchoconstriction'. However, this compound, they noted, did not block the pressor response to adrenaline and noradrenaline and the contraction of nictitating membrane by adrenaline or by stimulation of preganglionic nerve. Uterine contraction was unchanged with dichloroisoproterenol. The combined use of dibenamine and dichloroisoproterenol blocked both pressor and depressor responses of adrenaline. Moran and Perkins (1958) demonstrated that dichloro-isoproterenol selectively blocked the cardiac positive inotropic and chronotropic effects of adrenergic stimuli. Thus, support was given to the hypothesis of Ahlquist that the actions of sympathomimetic amines are mediated by two types of receptors and it became clear that each of them could be blocked by a different blocking agent.

The decade following the development of the first beta-adrenergic receptor blocking agent has seen the introduction of a number of newer agents and accumulation of a multitude of published data describing their pharmacological properties and clinical uses. These include pronethalol (Black and Stevenson, 1962), 1-(4'nitro-

phenyl [-2-isopropylamino-ethanol (INPEA)] (Teotino, Friz, Steis and Della Bella, 1963), propranolol (Black, Crowther, Shanks, Smith and Dornhorst, 1964), MJ-1999 (Lish, Weikel and Dungan, 1965), ICI 45,763 (KO 592) (Shanks, Wood, Dornhorst and Clark, 1966), 39089/Ba (oxprenolol) (Wilson and Turner, 1967), H 56/28 (Alprenolol) (Johnsson, Norrby, Solvell and Ablad, 1966) and butidrine (Marchetti, Merlo and Noseda, 1966). The blocking activity of these compounds is similar qualitatively in that they block all beta receptors, but differs quantitatively. The anticipated pharmacological response to blockade of beta-adrenergic receptor stimulation by these compounds are listed in Table I. Another group of compounds, which block some but not all beta receptors, has also emerged. These compounds include N-isopropyl-methoxamine (Levy, 1964) which blocks beta receptors in the rat uterus; N-tertiary butylmethoxamine (Levy, 1966a), and dimethyl isopropyl-methoxamine (Levy, 1966b) which blocks beta receptors in the rat uterus, canine intestine and peripheral blood vessels; and ICI, 50, 172 (AY-21,011) (Dunlop and Shanks, 1968) which selectively blocks beta receptors in the heart. In addition, studies on the biological properties of the optical isomers of pronethalol, propranolol and alprenolol have revealed that the levo isomers have greater potency than the racemic mixtures and that the dextro isomers have only negligible beta-adrenergic blocking activity (McInerny, Gilmour and Blinks, 1965; Howe, 1963; Barrett and Cullum, 1968; Ablad, Brogard and Ek, 1967).

In addition to the beta-adrenergic receptor blocking activity, most of these compounds have other pharmacological properties which are

Table I

Classification and responses of various effector organs to adrenergic stimuli and anticipated responses to beta-blockade.

CLASSIFICATION AND RESPONSES OF VARIOUS EFFECTOR ORGANS TO ADRENERGIC STIMULI AND ANTICIPATED RESPONSES TO BETA-BLOCKADE

EFFECTOR ORGAN	RECEPTOR TYPE	RESPONSE TO STIMULATION	RESPONSE TO BLOCKADE OF BETA RECEPTOR STIMULATION
HEART:			
Sinoatrial node	Beta	Increase in heart rate	Decrease in heart rate
Atrioventricular node	Beta	Increase in conduction velocity	Decrease in conduction velocity
Atria	Beta	Increase in contractility	Decrease in contractility
Ventricles	Beta	Increase in contractility	Decrease in contractility
SMOOTH MUSCLE:			
Blood vessels to skeletal muscle	(1) Alpha (2) Beta	(1) Contraction (constriction) (2) Relaxation (dilatation)	No direct effect Contraction
Blood vessels to skin & mucosa	Alpha	Contraction	No direct effect
Bronchial muscle	Beta	Relaxation	Contraction
GASTROINTESTINAL TRACT:			
Motility:			
Stomach	Beta	Decrease	Increase
Intestine	Alpha & Beta	Decrease	Increase
Sphincters:			
Stomach	Alpha	Contraction	No effect
Intestine	Alpha	Contraction	No effect
URINARY BLADDER:			
Detrusor	Beta	Relaxation	Contraction
Trigone & sphincter	Alpha	Contraction	No effect
EYE:			
Radial muscle; iris	Alpha	Contraction (mydriasis)	No effect
Ciliary muscle	Beta	Relaxation	Contraction
METABOLIC EFFECTS:			
Liver (Glycogenolysis)	Alpha	Increase	No effect
Muscle, skeletal & Cardiac (Glycogenolysis)	Beta	Increase	Decrease
Pancreas			
Insulin formation	Beta	Increase	Decrease
Insulin release	Alpha	Decrease	No effect
Lipid-containing cells			
Lipolysis	Beta	Increase	Decrease

Modified from Epstein, S.E. and Braunwald, E. (1966).

potentially of significance either clinically or in relation to the mode of action of the drugs. Fitzgerald (1969) classified these drugs according to their beta-adrenergic blocking property, membrane-stabilizing property and intrinsic sympathomimetic activity (Table II).

Dichloroisoproterenol, because of its prominent intrinsic sympathomimetic action, has not found a place in clinical use. Pronethalol, with very little intrinsic sympathomimetic effect and membrane effect, was used in many patients. It was withdrawn from clinical use because it caused occasional lymphosarcomas in mice (Paget, 1963). Propranolol which has no intrinsic sympathomimetic action but has membrane activity, has been used most extensively. This drug has main application in angina pectoris, digitalis- and non-digitalis-induced cardiac arrhythmias, idiopathic hypertrophic subaortic stenosis and selected cases of hypertension, pheochromocytoma and hyperthyroidism (Epstein and Braunwald, 1966).

Snow (1965) used propranolol, 10-20 mg eight-hourly in 45 patients with acute myocardial infarction within the preceding 24 hours. The mortality in this group was 16 percent compared with 35 percent mortality in a control group of 46 patients. He concluded that propranolol may have acted by reducing the demand for oxygen of the myocardium and also by inhibiting dangerous arrhythmias. Unfortunately, no electrocardiographic monitoring was done in these patients. The enthusiasm of Snow was not shared by others. Other studies initially suggested that propranolol was, in fact, detrimental in patients with acute myocardial infarction.

Clausen, Felsby, Schonau, Jorgensen, Lyager Nielsen, Roin

Table II

Classification of the beta-adrenergic receptor blocking drugs.

CLASSIFICATION OF BETA-BLOCKING DRUGS

	Drug	Beta-Blocking	Membrane Activity	I.S.A.	Selectivity	Potency
A.	D.C.I.	+	+	+++	-	0.1
	Pronethalol	+	+	++	-	0.1
	ICI. 45,763	+	+	++	-	1.0
	Oxprenolol	+	+	++	-	2.0
	Alprenolol	+	+	++	-	1.0
B.	Propranolol	+	+	-	-	1.0
	Butidrine	+	+	-	-	0.1
C.	INPEA	+	-	+	-	0.04
D.	Sotalol (MJ.1999)	+	-	-	-	0.1
E.	ICI. 50,172 (AY. 21,011) (Practolol)	+	-	+	Cardio-selective	0.5
	Butoxamine	+	-		Metabolic Periph. Vasc.	
F.	*d*-Propranolol	-	+	-	-	0.01
	l-Propranolol	+	+	-	-	2.0
	d-Alprenolol	-	+	-	-	
	l-Alprenolol	+	+	+	-	2.0

Adopted from Fitzgerald, J. D. (1969).

and Strange (1966) could not find any prophylactic effect of propranolol, given 10 mg four times a day, with regard to mortality, arrhythmia and complications following myocardial infarction. Barber, Murphy and Merrett (1966) using 40 mg every six hours, found no significant decrease in mortality. There were no serious side effects and the incidence of heart failure was similar in the control and the treated groups. Balcon, Jewitt, Davies and Oram (1967), in a double blind study by using propranolol in an oral dose of 20 mg every six hours, could not find any significant difference in mortality, arrhythmia, heart failure and shock between the control and the treated groups. They, however, noted that the incidence of hypotension and sinus bradycardia was significantly increased in the treated group. Ledwich (1968) failed to note any beneficial or detrimental effect of propranolol following myocardial infarction. Norris, Caughey and Scott (1968) demonstrated in a large number of patients that the mortality rate in propranolol-treated and control group with acute myocardial infarction was not significantly different. They, however, found no evidence that propranolol caused morbidity from cardiac failure and hypotension. They concluded that the tendency of oral propranolol in doses up to 80 mg per day to exacerbate heart failure or hypotension has been overemphasized. They did not deny its usefulness in larger doses or given parenterally in some specific cardiac arrhythmia following myocardial infarction. Sloman, Robinson and McLean (1965) reported two patients with ventricular fibrillation following infarction in whom treatment with propranolol allowed sinus rhythm to be maintained following DC-counter-shock after other drugs failed although only one case survived. Ikram (1968) described four cases of

persistent ventricular fibrillation, complicating acute myocardial infarction, which reappeared repeatedly despite usual management. With intravenous administration of one mg of propranolol, there was long-lasting reversion to normal rhythm in three patients. The one patient who did not respond had a massive infarction involving two-thirds of the left ventricle.

The number of investigations involving the use of propranolol or any of the other beta-adrenergic blocking agents, to determine if this group of drugs has any effect on ventricular fibrillation and death *soon* after acute coronary occlusion, is very limited. Weiser, Reich, Panagopoulos, Armellini, Gelman and Kaley (1966) reported hemodynamic effects of propranolol with acute myocardial infarction. Intravenous injection of propranolol (0.25 mg/kg) given 10 minutes after ligation of the anterior descending branch of the left coronary artery decreased cardiac output progressively, more than was seen following coronary ligation alone (-22 percent to -38 percent). The mean aortic pressure was maintained. Propranolol decreased the heart rate (-16 percent) and negated the increase seen in the untreated group (+15 percent). Systemic vascular resistance was increased (+24 percent). The left ventricular work decreased in proportion to the decrease in cardiac output. Pentecost and Austen (1966) reported the influence of propranolol in acute myocardial infarction in anesthetized open-chest dogs. Six out of seven control dogs died from ventricular fibrillation compared with one out of seven dogs receiving propranolol, 0.08 mg/kg, and three out of six dogs receiving propranolol, 0.2 mg/kg intravenously just prior to the ligation of the circumflex branch of the left coronary artery. Proger, Sharma and Naimi (1966) also reported

the beneficial effect of propranolol in experimental occlusion of the anterior descending branch of the left coronary artery in anesthetized dogs. The experimental group was given an intravenous injection of propranolol (0.31 mg/kg) five minutes prior to ligation. This was followed by slow infusion of the drug for 100 minutes "calculated to maintain 100 percent blockade". Six out of 10 control animals died of ventricular fibrillation within 22 minutes of ligation. Only two out of 10 in the experimental group died during the period of observation. There were fewer ventricular ectopic beats in the treated group. Propranolol in doses of 0.2 to 0.3 mg/kg intravenously led to complete suppression of arrhythmias in six out of seven anesthetized open-chest dogs following ligation of the anterior descending artery. In three of these dogs, subsequent ligation of a second branch of the left coronary artery and infusion of adrenaline up to 8 µg/min did not induce further arrhythmia (Ceremuzynski *et al.*, 1969).

Kaumann and Aramendia (1968), using MJ-1999 in pentobarbital anesthetized dogs, demonstrated that this drug prevented ventricular fibrillation following the ligation of the anterior descending branch of the left coronary artery. This protection was afforded by a dose of 10 mg/kg and not by 0.5 mg/kg body weight. They also showed that coronary occlusion still produced ventricular fibrillation in animals which received reserpine, 0.5 mg/kg 24 hours prior to the occlusion under anesthesia. However, MJ-1999 given at a dose of 10 mg/kg prevented ventricular fibrillation in dogs pre-treated with reserpine. These investigators suggested that the antifibrillatory effect of this drug may be independent of its beta receptor blocking effect.

They noted that MJ-1999 has the property of prolonging the duration of action potential and that the antifibrillatory action may be related to this property. However, it is well established that MJ-1999 does not have the membrane stabilizing action possessed by propranolol (Somani and Lum, 1965; Somani, Fleming, Chan and Lum, 1966; Lish and Soloff, 1968; Levy, 1968).

Only a few studies have been published in which the effect of AY-21,011 was determined following coronary occlusion. Ceremuzynski *et al.* (1969) gave 0.5 to 1.0 mg/kg of AY-21,011 to only three anesthetized, open-chest dogs which had developed severe ventricular arrhythmia (more than 20 ectopic beats per min) following coronary occlusion. This led to complete restoration of the sinus rhythm in two animals and reduced the number of ectopic beats in the third. Jewitt, Burgess and Shillingford (1970), while studying the circulatory effects of AY-21,011 in patients with acute myocardial infarction, noted that the frequency of ventricular extrasystoles was reduced in two out of five patients receiving five mg, and in five out of 10 patients receiving 25 mg of this drug intravenously.

We are unaware of any published report on the use of *dl*-propranolol, MJ-1999 and AY-21,011 in the prevention of ventricular fibrillation *soon* after experimental coronary occlusion in *conscious* animals. We are also unaware of any attempt to elucidate whether the antifibrillatory action of *dl*-propranolol in occlusion fibrillation is due to its beta-adrenergic blocking effect or its nonspecific local anesthetic, membrane-stabilizing and the so-called "quinidine-like" effect. Furthermore, we have not been able to find any published

investigation in which continuous monitoring of every ventricular beat was continued, for a period of even one hour following experimental coronary occlusion.

METHODS AND MATERIALS

I. Determination of equiactive doses of *dl*-propranolol, MJ-1999 and AY-21,011.

A. Determination of dose-response curves for *dl*-propranolol- and MJ-1999-blockade of isoproterenol-induced tachycardia.

Healthy mongrel dogs, weighing between five and 18 kg, were used for this study. All experiments were carried out on conscious animals. The procedures were performed in an air-conditioned operating room at temperatures between 21° and 24° C.

The animal was gently restrained on an operating table. Under local anesthesia, using two percent lidocaine, a polyethylene catheter was placed in one of the veins of a limb and connected to an overhead bottle of normal saline by a connecting tube. All drugs were administered through this polyethylene catheter and flushed by running normal saline from the overhead bottle. An electrocardiogram was obtained from fine subcutaneous needles and was recorded on a Sanborn multichannel machine. The electrocardiogram was used to determine the heart rate.

After the dog had calmed down and the heart rate had stabilized, an electrocardiogram was recorded to determine the control heart rate. The dog was then challenged with isoproterenol during continuous electrocardiogram recording. In all experiments the challenging dose of isoproterenol was 1.5 μg/kg. A second challenging dose was given after the heart rate came back to control level, usually in 15 to 20 minutes. The mean of the

percent change of heart rate induced by these two isoproterenol challenges was taken as "control percent increase with isoproterenol".

Once the heart rate came back to control level, *dl*-propranolol or MJ-1999 was administered. The animal was then re-challenged with isoproterenol five minutes later and then every 10 minutes thereafter for five to six times. By using cross-over allocation, 23 experiments were performed on 10 dogs with *dl*-propranolol with doses from 0.05 mg/kg to 1.0 mg/kg. Each animal was thus exposed to two to three dose levels of *dl*-propranolol. Fifteen similar experiments were performed on eight dogs with MJ-1999 using doses from 0.1 mg/kg to 2.4 mg/kg.

B. Determination of dose-response curve for AY-21,011-blockade of isoproterenol-induced tachycardia.

This was done in a manner similar to that for *dl*-propranolol and MJ-1999 except for the fact that the study was done on conscious animals pre-treated with atropine. Atropine sulphate, 0.2 mg/kg, was given intravenously 10 minutes before commencing the study. Nine experiments were performed on five dogs with AY-21,011 in doses from 0.4 mg/kg to 6.4 mg/kg.

The percent blockade of isoproterenol-induced tachycardia by these drugs was calculated by the following formula:

100 x (Control percent increase with isoproterenol minus percent increase after beta-blocking drugs): (Control percent increase with isoproterenol).

C. Estimation of the dose of *d*-propranolol having equiactive membrane-effect as that of ED_{50} dose of *dl*-propranolol.

This was determined from the study of Barrett and Cullum (1968) using isolated frog sciatic nerve. These investigators immersed the nerve in a bath containing Ringer solution at room temperature. One end of the nerve was placed on a pair of stimulating electrodes and the other end on a recording electrode. The conducted compound action potential was displayed on an oscilloscope and the amplitude was measured. The doses of the optical isomers of propranolol necessary to produce a 50 percent reduction in the spike amplitude were determined.

II. Production of coronary occlusion in conscious dogs.

Adult healthy mongrel dogs of either sex weighing between 5 and 18 kg were used. Very young and old dogs were excluded. As with the experiments to determine the dose-response curves, all experiments were carried out in an air-conditioned operating room at temperatures between 21° and 24° C.

Experimental coronary occlusion was produced in two stages (Manning, McEachern and Hall, 1939). In the first stage the animal was prepared for the second stage. The dog was anesthetized with I.V. pentobarbital, 25-30 mg/kg. A preoperative electrocardiogram was recorded. The dog was then intubated and ventilated with room air using a Harvard pump respirator. With the dog lying on its right side, the left side of the chest was shaved, the skin was cleansed with tincture of iodine and the operative area was draped. The operation was done with proper aseptic precaution by

using mask and sterile gloves, gown and surgical instruments. The chest was opened by an incision through the fourth or fifth intercostal space. The pericardium was incised along a line anterior and parallel to the left phrenic nerve. A ligature was placed at the tip of the left atrial appendage to retract it, so that the area of the bifurcation of the left coronary artery into its anterior descending and circumflex branches was exposed. Staying as close to the anterior descending branch as possible, the most proximal part of the circumflex branch was freed from the epicardium and its bed on the myocardium. A No. 4 silk ligature was placed around the circumflex branch by using a hollow curved needle mounted on a probe handle. Passage of the silk cord through the hollow needle avoided bruises and trauma to the blood vessel. A loose half-knot was then made in the ligature making a wide loop around the artery leaving the vessel unoccluded. The ends of the long silk cord were then exteriorized through the pericardium and two separate stab wounds beyond the ends of the skin incision. Care was taken to see that these two exit points and the origin of circumflex branch were in a straight line. Sufficient cord was left loosely inside the chest to avoid tying off of the vessel by the dog's respiratory movements. The pericardium was not sutured back. The lungs were expanded to eliminate pneumothorax as much as possible and the chest was closed in layers. The exteriorized ends of the silk cord were secured to the skin by using extra sutures. The wound was sealed with gauze and collodion flexible. The dog was allowed

to breathe spontaneously and then returned to its cage.

The second stage constituted the experiment proper, and was carried out 48 to 72 hours after the first stage with the animal fully conscious. Each experiment was conducted with the dog lying on its right side with all four limbs tied down while an assistant pacified the animal. With two percent lidocaine infiltration, the right femoral artery and vein were isolated. An NIH catheter inserted into the femoral artery, firmly secured by cotton ties, was used for collection of arterial blood sample and for recording blood pressure through a Statham P23 Db transducer. Another catheter was placed into the femoral vein and connected to an overhead bottle of normal saline. All drugs were administered through the venous catheter and immediately flushed by running normal saline from the overhead bottle. Electrocardiogram (Lead II) was obtained from fine subcutaneous needles and was recorded by a direct writer Sanborn (Model 67-1600) multichannel machine simultaneous with the blood pressure. The electrocardiogram was also recorded on magnetic tape using a Sony tape recorder for later detailed analysis of arrhythmias. The first arterial blood sample for blood gases and pH was drawn and the catheter flushed with normal saline containing 20,000 U.S.P. units of heparin per 500 ml. Control electrocardiogram and blood pressure of the calm conscious animal were recorded by the direct writer. The control animal then received 2-3 ml normal saline through the venous catheter which was then flushed by running normal saline from the overhead bottle. The treated animal received an

appropriate amount of the assigned drug instead of normal saline in exactly the same manner. Five minutes after the administration of saline or the drug, a second arterial blood sample was taken, and the electrocardiogram and blood pressure were recorded again. At this moment the tape recorder was started in order to record the electrocardiogram continuously until the end of the experiment. With the direct writer continuously recording simultaneous electrocardiogram and blood pressure, the exteriorized ends of the silk cord were pulled uniformly by winding the cord around the index finger of the operator to occlude the circumflex branch totally. Traction on the cord was maintained for a minimum of one minute or until the acute changes in T-wave and ST-segment in the electrocardiogram were evident. The direct writer was used continuously for the first 10 minutes after coronary occlusion and then intermittently at 15 minutes of occlusion and every 15 minutes thereafter for a period of two hours or less, if the dog succumed to coronary occlusion. The tape recorder, however, recorded continuously the electrical events of the heart throughout the period of observation. A third sample of arterial blood was taken 10 minutes after the coronary occlusion or, if the dog died, immediately following its demise. After the experience of completing 65 experiments the electrocardiographic monitoring was continued for only one hour while the animal was still observed for mortality for a total of two hours following the coronary ligation. The last two arterial blood sample collections were also discontinued at this stage in the study

and only the first sample collection was continued.

If the animal died during the two hour period of observation, an autopsy was performed immediately to determine the exact location of the ligature and the adequacy of ligation. The heart was removed with its attached great blood vessels. The circumflex branch was dissected out for a length of about 1.5 cm beyond the occlusion and divided at the distal end of the mobilized portion. The nozzle of a water-filled syringe was firmly secured to the ascending aorta with the nozzle directed towards the heart. Water was then forced out of the syringe against the aortic valve, the closure of which made the injection of the coronary arteries possible. The cut end of the circumflex branch was observed for leakage. The circumflex branch proximal to the ligature was then dissected towards the main coronary artery and its division. The distance of the ligature from the origin of the circumflex branch was measured. Observation was made to see if any branch of the circumflex artery escaped ligation. These animals were classed as "two-hour" deaths.

If the dog survived the two hour period of observation, it was returned to its cage after removal of the arterial and venous catheters and closure of the skin incision. No restriction was imposed on its activity or feeding. If the dog died during the first 24 hours autopsy was performed to note the adequacy and location of coronary ligation. The presence or absence of gross myocardial infarction was noted. These animals were classed as "24-hour" deaths.

Animals surviving 24 hours after coronary occlusion were brought back to the operating room. An electrocardiogram was recorded to note the evidence for definite myocardial infarction. The dog was then sacrificed using i.v. pentobarbital sodium 100 mg/kg. The heart was removed and examined for the adequacy and location of ligation as outlined above. In addition, the presence and extent of gross myocardial infarction was noted.

III. Criteria for acceptance into the study.

In the "2-hour" and "24-hour" death groups, only those animals showing no leakage through the ligated artery and only those cases where the ligature was placed before the circumflex artery gave off any branch, were included in this study. The presence of gross myocardial infarction was not necessary for inclusion in the 24-hour death group in the study. However, its presence or absence was noted to get some idea about the time of death. In the animals surviving the 24-hour period, two additional criteria were needed. These were electrocardiographic evidence for definite myocardial infarction and the presence of gross myocardial infarction involving the postero-lateral surface of the left ventricle extending to the posterior part of the interventricular septum.

IV. Allocation of dogs to different experimental groups.

The method of allocation of the animals to the different groups requires some chronological description. Initially, the study was begun with three experimental groups:

1. Control - these animals received 2-3 ml normal saline.
2. *dl*-propranolol, ED_{50} - these animals received *dl*-propranolol at the ED_{50} dose determined above.
3. *dl*-propranolol, ED_{100} - these dogs received *dl*-propranolol at the ED_{100} dose determined above.

An attempt was made to allocate the animals to the different groups by matching their weights as closely as possible. The experiments were done simultaneously, not sequentially; i.e., for each control dog, there was one experiment done on one dog in each of the other two groups. The group receiving *dl*-propranolol in the ED_{100} dose was not used after 10 experiments of this category were completed. After 38 experiments, one new experimental group was added by using the ED_{50} dose of MJ-1999. From this stage, three experiments were done with the ED_{50} dose of MJ-1999 for each of the control and *dl*-propranolol-ED_{50} groups. After 30 more experiments; i.e., at the completion of a total of 68 experiments, four additional experimental groups were added. Again, to make a balanced study, for one experiment in each of the control, *dl*-propranolol ED_{50} and MJ-1999 ED_{50} groups, three to four experiments were done on each of the four newly added experimental groups. The newly added experimental groups were:

1. A group receiving the ED_{100} dose of MJ-1999.
2. A group receiving the *d*-propranolol in dose equivalent to the membrane-effect possessed by the ED_{50} dose of propranolol (data from Barrett and Cullum, 1968).

3. A group receiving a combination of the ED_{50} dose of MJ-1999 and the estimated dose of *d*-propranolol as in the previous group.
4. A group receiving the ED_{50} dose of AY-21,011.

At the completion of this study 141 acceptable experiments, out of a total of 154, were performed on eight experimental groups as follows:

1. Control group----------25 dogs
2. *dl*-propranolol, ED_{50}----------25 dogs
3. *dl*-propranolol, ED_{100}----------10 dogs
4. MJ-1999, ED_{50}----------25 dogs
5. MJ-1999, ED_{100}----------12 dogs
6. *d*-propranolol----------16 dogs
7. MJ-1999, ED_{50} + *d*-propranolol----------12 dogs
8. AY-21,011, ED_{50}----------16 dogs

Total 141 dogs

V. Analysis of blood gas, electrocardiogram and arrhythmias.

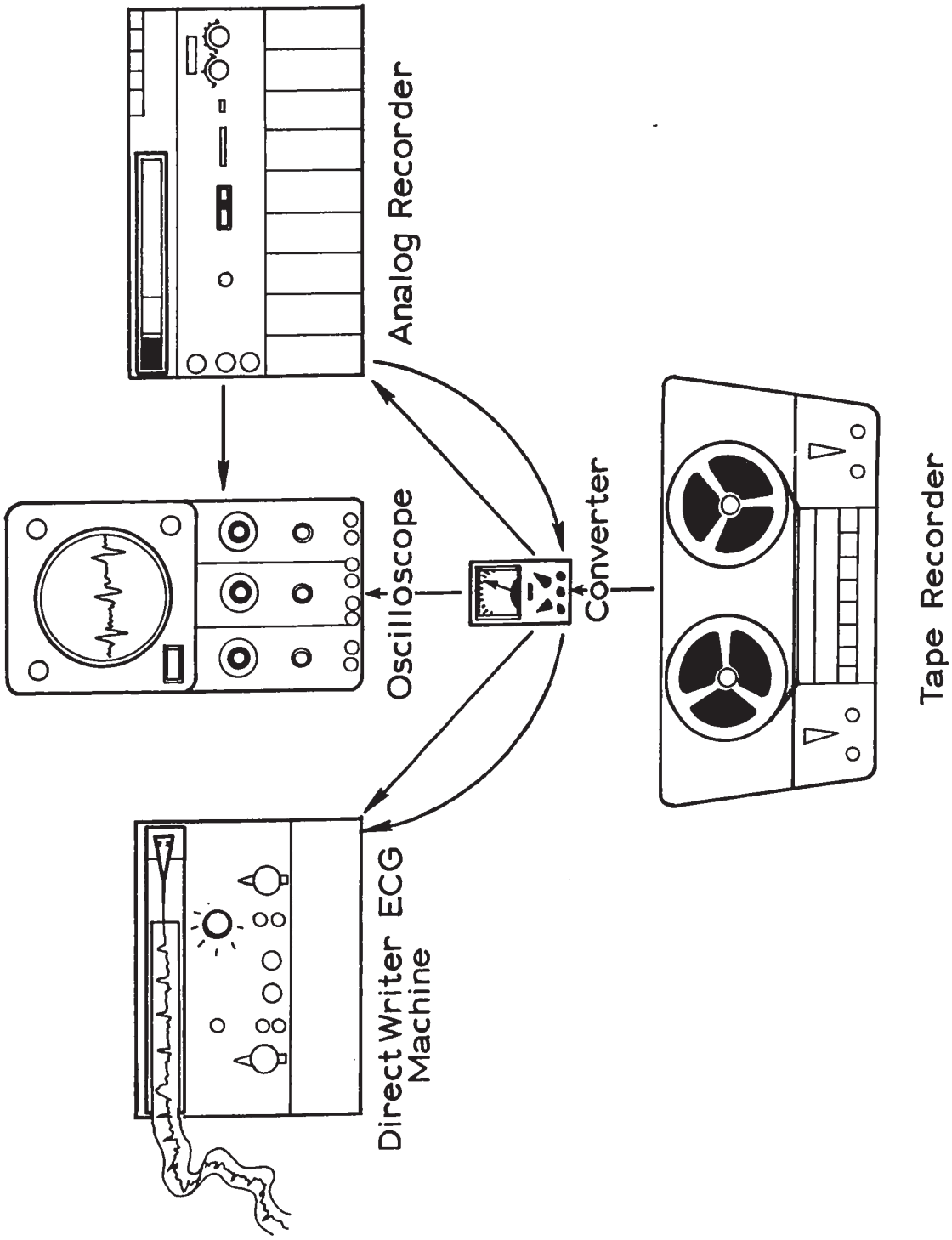
The arterial blood sample was analyzed for pH, pO_2, pCO_2 and O_2-saturation by the personnel of the cardio-pulmonary laboratory of Victoria Hospital, London, Canada.

The direct writer data of blood pressure and electrocardiogram were used to measure the blood pressure at various times during the period of observation. Electrocardiogram was used to determine the heart rate and PR interval during the control period, after saline or drug administration and after

Figure 1

The system used for detailed analysis of arrhythmia from magnetic tape.

(Converter = Analog Data Converter)


Analog Recorder
Oscilloscope
Converter
Tape Recorder
Direct Writer ECG Machine

coronary occlusion. In addition, the number of ventricular ectopic beats and ventricular tachycardias were counted. T-wave and ST-segment changes and other changes in the electrocardiogram were also noted.

Detailed analysis of arrhythmia from the magnetic tape was done by the system depicted in Figure 1. The magnetic tape was played back using a Phillips tape recorder with attached arrangement for fluorescent visual display of electrocardiogram on a Dumont 401B oscilloscope after passing it through an A to D converter. A Hewlett-Packard electrocardiogram machine in the system allowed direct write-out of any segment of taped electrocardiogram, if needed for more careful analysis. Phillips Analog-7 recorder allowed re-recording and play-backs at slower than recorded rate, when needed. Nomenclature and criteria for electrocardiographic diagnosis were adopted from the recommendations by the Criteria Committee of the New York Heart Association (1964).

VI. Statistical Methods

The slopes of the regression equations for *dl*-propranolol, MJ-1999 and AY-21,011 were compared by F-test. When any two of these slopes were compared, student's t-test was used.

The characteristic features of experimental animals, e.g., pH, pO_2, pCO_2 and O_2-saturation, were compared with those of normal non-thoracotomized dogs by student's t-test for the significance of the difference.

The significance of the effect of normal saline or drugs

on the heart rate, blood pressure and the PR interval was tested by student's t-test using paired data. The mean changes produced in these parameters by normal saline or drugs were then subjected to overall analysis of variance to test the significance of the difference between these changes as well as to test the significance of the effect of this group of drugs as a whole compared to that of normal saline. The immediate effects of coronary occlusion on the heart rate and blood pressure were also subjected to similar analysis.

The Chi-Square method was used to test the significance of the difference between the mortality rates in the various experimental groups. The difference in the number of experimental animals exhibiting or not exhibiting a particular cardiac arrhythmia was also tested for significance by this method.

For numerical analysis, the ventricular ectopic beats were expressed in terms of their frequency per dog per minute during the three 5-minute periods following coronary occlusion. Analysis of variance was used to test the significance of the difference between their frequency in various experimental groups for each of these periods. In addition, student's t-test was used to compare the frequency of the ectopic beats between the control and each of the various treatment-groups.

The difference between each of these parameters tested was considered significant only when the p values were less than 0.05.

VII. Materials.

The following drugs were used:

Lidocaine (Xylocaine^{R}) hydrochloride, 2 percent, U.S.P., Astra Pharmaceuticals.

Isoproterenol (Isuprel^{R}) hydrochloride, Winthrop Laboratories.

dl-propranolol (Inderal^{R}) hydrochloride, Ayerst Laboratories.

d-propranolol hydrochloride, Ayerst Laboratories.

AY-21,011 (IC150172, Practolol^{R}), Ayerst Laboratories.

(The above three drugs were kindly supplied by Dr. R. O. Davies of Ayerst Laboratories, Montreal, Canada.).

MJ-1999 (Sotalol^{R}) hydrochloride, Mead Johnson & Co.

(This drug was kindly supplied by Dr. G. R. McKinney of Mead Johnson Research Center, Evansville, Indiana.).

Atropine sulphate, Shawinigan Company.

Veterinary pentobarbital (Nembutal) sodium, U.S.P., 60 mg/ml, Abbott Laboratories Limited.

Pentobarbital (Euthansol) sodium, 180 mg/ml, W.E.Saunders Ltd.

Heparin sodium, 10,000 U.S.P. Units per ml, Riker Pharmaceutical Co. Ltd.

dl-propranolol, *d*-propranolol, MJ-1999, AY-21,011 and atropine were dissolved in normal saline at the required concentrations. All doses are expressed in terms of the salt used.

Figure 2 shows the structures of the beta-adrenergic receptor blocking drugs used in the present study.

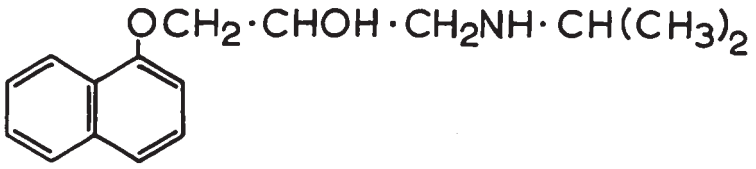
Figure 2

Structural formulas of beta receptor blocking agents used in this study.

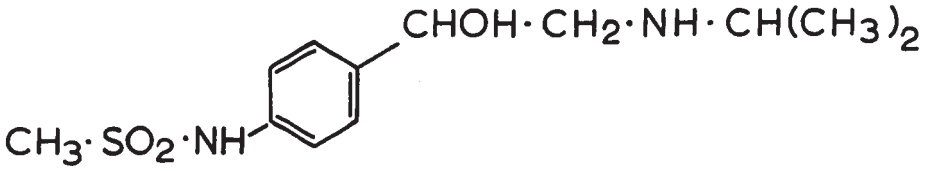
A. Propranolol [1-(Isoproprylamino)-3-(1-naphthyloxy)2-propanol]

B. MJ-1999 [4-(2-isopropylamino-1-hydroxyethyl) methanesulphonanilid]

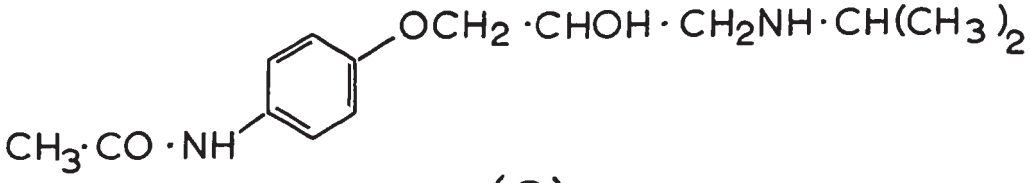
C. AY-21,011 [4-(2-hydroxy-3-isopropyl-aminopropoxy) acetanilide]


OCH2·CHOH·CH2NH·CH(CH3)2

(A)


CHOH·CH2·NH·CH(CH3)2
CH3·SO2·NH

(B)


OCH2·CHOH·CH2NH·CH(CH3)2
CH3·CO·NH

(C)

RESULTS

I. Determination of suitable doses of beta-blocking drugs.

A. Dose-response curves for *dl*-propranolol, MJ-1999 and AY-21,011*

The following are the regression equations for these compounds based on the data presented in Appendix I:

dl-propranolol : Y = 98.98 + 48.04X
(Standard Error of the estimate = 4.44)

MJ-1999 : Y = 78.83 + 39.09X
(Standard Error of the estimate = 1.64)

AY-21,011 : Y = 45.06 + 30.75X
(Standard Error of the estimate = 0.41)

The dose-response curves derived from the above equations are shown in Figure 3. The difference between the slopes of the three dose-response curves is significant ($p < 0.05$).

The regression equations yielded values of 0.0956 mg/kg and 0.183 mg/kg for the ED_{50} doses of *dl*-propranolol and MJ-1999 respectively. Nevertheless, the more convenient values of 0.1 mg/kg and 0.2 mg/kg for *dl*-propranolol and MJ-1999 respectively, were used. The calculated percent block caused by these doses were 50.94 for the former and 51.51 for the latter. Likewise, for ED_{100} doses of 1.0 mg/kg instead of 1.05 mg/kg for *dl*-propranolol and 3.2 mg/kg instead of 3.5 mg/kg for MJ-1999, were used in the present study. The estimated percent block of isoproterenol-induced tachycardia by these doses of *dl*-propranolol and MJ-1999 were 98.98 and 98.58 respectively. The error so involved was considered negligible.

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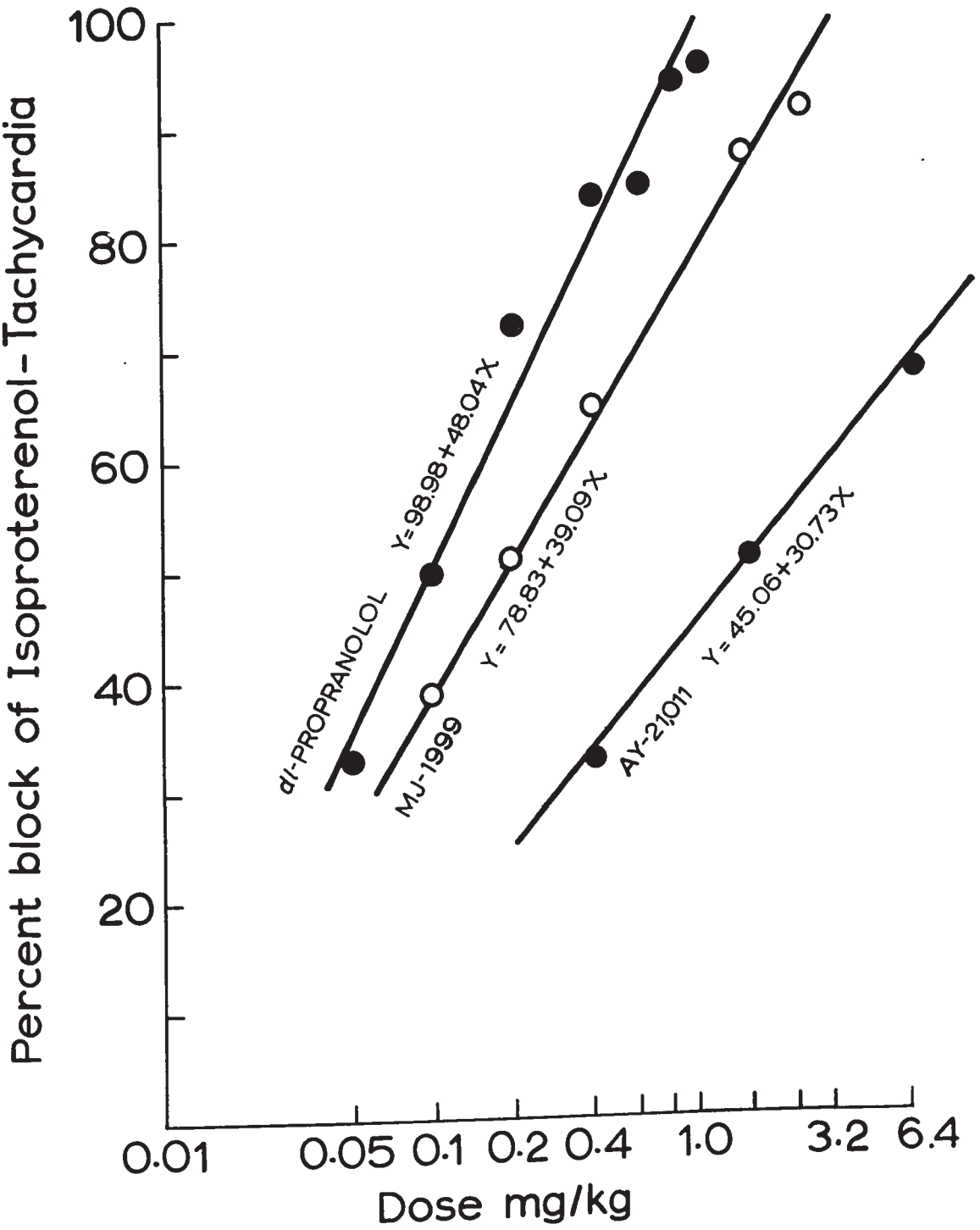
* Where X = log dose, Y = mean percentage block

Figure 3

Dose-response curves for *dl*-propranolol, MJ-1999 and AY-21,011.


Percent block of Isoproterenol-Tachycardia
100
80
60
40
20
0.01
0.05
0.1
0.2
0.4
1.0
3.2
6.4
Dose mg/kg
d/-PROPRANOLOL
Y=98.98+48.04X
MJ-1999
Y= 78.83+39.09X
AY-21011
Y=45.06+30.73X

For AY-21,011, only the calculated ED_{50} dose of 1.4 mg/kg was used in the present investigation.

B. Dose of *d*-propranolol having equiactive local anesthetic effect as that of the ED_{50} dose (0.1 mg/kg) of *dl*-propranolol.

Based on the studies of Barrett and Cullum (1968), the mean concentrations (± standard error) of *dl*-propranolol, *d*-propranolol and *l*-propranolol which produced 50 percent reduction in the spike amplitude were 28.5 ± 1.3, 20.6 ± 1.6 and 20.2 ± 0.5 µg/ml respectively. The calculated dose of *d*-propranolol, from these data, having the same membrane effect as that of 0.1 mg of *dl*-propranolol was thus 0.0723 mg.

II. Characteristic features of the experimental animals.

The mean body weight (± standard error) of the experimental animals was 9.6 ± 0.26 kg. The arterial blood analysis showed the following:

Mean pH	7.34 ± 0.00
Mean pO_2	77.03 ± 0.56 mm Hg
Mean pCO_2	36.40 ± 0.48 mm Hg
Mean O_2-saturation	89.7 ± 0.44 percent

The mean pH, pO_2, pCO_2 and O_2-saturation of the arterial blood of nine non-thoracotomized normal dogs were 7.35 ± 0.00, 78.67 ± 1.42, 35.2 ± 0.95 and 88.4 ± 1.33 respectively, and were not significantly different from those of the experimental group ($p > 0.2$).

III. The effects of beta-blocking drugs in conscious dogs.

A. General Observations.

No unusual general effects, e.g., vomiting, diarrhea, excitement, tremor, convulsion, stupor or shortness of breath were noted following the administration of the drugs used in this study.

B. Heart Rate.

As shown in Table III and Figure 4 (from the point C to the point O), the mean heart rate was decreased by all beta-blocking drugs but not altered after administration of normal saline. Analysis of the changes in the heart rate as paired data showed that these changes were significant for some treatments and not for others. The magnitude of the changes was of such a degree that an overall analysis of variance using the mean changes demonstrated them to be not significant. On the other hand, there was a consistent pattern of a fall in heart rate after administration of the beta-blocking drugs. When the changes in the heart rate caused by all the treatments taken together were compared with the change after administration of normal saline, the difference was significant ($p < 0.01$).

C. Blood Pressure.

Treatment with the beta-blocking drugs caused an increase in both systolic and diastolic blood pressures (Tables IV and V, Figures 5 and 6, from C to O). These changes, analyzed as paired data, were significant in some treatment groups and not in others. Overall analysis of variance using the mean changes showed them not to be significant. As with the change in the

TABLE III

The effect of various drugs compared to normal saline injection on the mean heart rate of conscious dogs. The p values shown were determined by student's t-test using paired data. The difference of the changes between the eight groups by overall analysis of variance is not significant; the difference in the change in Group 1 compared with Groups 2 through 8, taken together, is significant ($p < 0.01$).

The effect of various drugs compared to normal saline injection on the mean heart rate of conscious dogs.

MEAN HEART RATE ± S.E.M.				
Drug or Saline	Before	After	Mean Change	p value of the difference
(1) Normal saline	121.9 ± 5.0	123.2 ± 5.0	+ 1.3 ± 2.2	> 0.5
(2) *dl*-propranolol 0.1 mg/kg	107.4 ± 4.8	99.6 ± 4.6	- 7.8 ± 2.8	< 0.01
(3) *dl*-propranolol 1.0 mg/kg	122.5 ± 7.3	110.9 ± 5.6	-11.6 ± 3.8	< 0.02
(4) MJ-1999 0.2 mg/kg	118.7 ± 2.7	112.8 ± 2.8	- 6.0 ± 2.0	< 0.01
(5) MJ-1999 3.2 mg/kg	95.4 ± 6.5	89.6 ± 4.4	- 5.8 ± 5.3	> 0.3
(6) *d*-propranolol 0.0723 mg/kg	117.3 ± 9.5	112.3 ± 9.5	- 5.1 ± 5.1	> 0.3
(7) MJ-1999 0.2 mg/kg + *d*-propranolol 0.0723 mg/kg	114.4 ± 5.0	111.0 ± 4.3	- 3.4 ± 3.1	> 0.2
(8) AY-21,011 1.d mg/kg	109.6 ± 7.1	105.8 ± 7.2	- 3.8 ± 2.6	> 0.1

Figure 4

Time-course of the mean heart rate in various groups.

C = Base line value, O = coronary occlusion, C to O = effect of normal saline or drug.

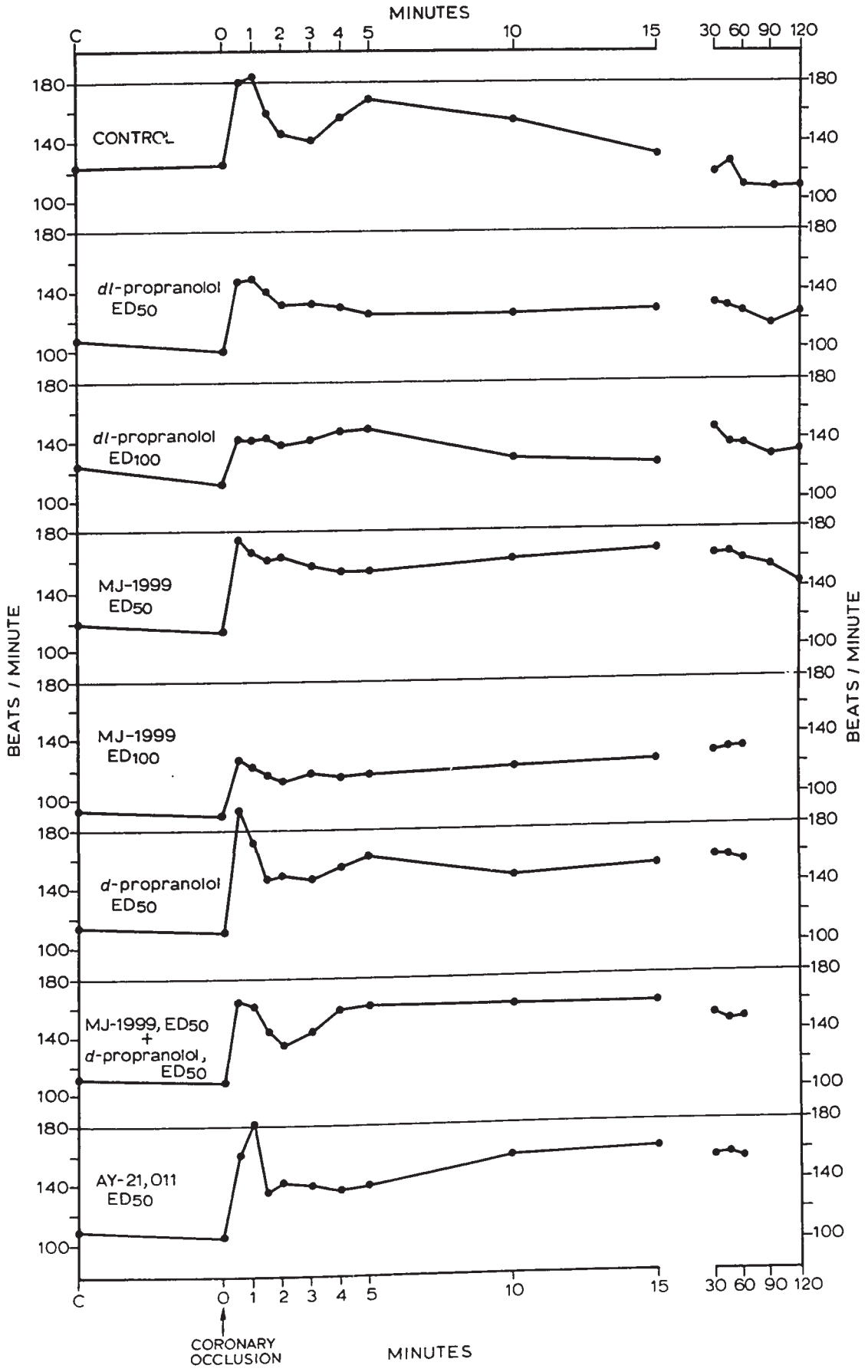

MINUTES
C
0 1 2 3 4 5
10
15
30 60 90 120
CONTROL
dl-propranolol ED50
dl-propranolol ED100
MJ-1999 ED50
MJ-1999 ED100
d-propranolol ED50
MJ-1999, ED50 + d-propranolol, ED50
AY-21,011 ED50
180
140
100
BEATS / MINUTE
CORONARY OCCLUSION
MINUTES

TABLE IV

The effect of various drugs compared to normal saline on the mean systolic blood pressure in conscious dogs. The p values shown were determined by student's t-test using paired data. The difference of the changes between the eight groups by overall analysis of variance is not significant; the difference in the change in Group 1 compared with Groups 2 through 8, taken together, is significant ($p < 0.05$).

The effect of various drugs compared to normal saline on the mean systolic blood pressure in conscious dogs.

MEAN SYSTOLIC BLOOD PRESSURE (mm Hg) ± S.E.M.				
Drug or Saline	Before	After	Mean Change	p value of the difference
(1) Normal saline	153.4 ± 4.8	154.3 ± 4.3	+ 0.9 ± 2.0	> 0.5
(2) *dl*-propranolol 0.1 mg/kg	154.9 ± 4.0	164.8 ± 4.2	+ 9.9 ± 1.7	< 0.001
(3) *dl*-propranolol 1.0 mg/kg	155.0 ± 6.7	161.4 ± 5.6	+ 6.4 ± 2.4	< 0.05
(4) MJ-1999 0.2 mg/kg	147.1 ± 3.7	151.8 ± 3.9	+ 4.6 ± 1.9	< 0.05
(5) MJ-1999 3.2 mg/kg	158.5 ± 6.0	161.0 ± 7.4	+ 2.5 ± 4.7	> 0.5
(6) *d*-propranolol 0.0723 mg/kg	164.0 ± 6.7	168.5 ± 8.1	+ 4.5 ± 3.3	> 0.1
(7) MJ-1999 0.2 mg/kg + *d*-propranolol 0.0723 mg/kg	164.3 ± 5.5	170.0 ± 6.8	+ 5.7 ± 4.4	> 0.2
(8) AY-21,011 1.4 mg/kg	162.1 ± 5.6	168.6 ± 5.8	+ 6.5 ± 3.4	> 0.1

TABLE V

The effect of various drugs compared to normal saline on the mean diastolic blood pressure in conscious dogs. The p values shown were determined by student's t-test using paired data. The difference of the changes between the eight groups by overall analysis of variance is not significant; the difference in the change in Group 1 compared with Groups 2 through 8, taken together, is significant ($p < 0.05$).

The effect of various drugs compared to normal saline on the mean diastolic blood pressure in conscious dogs.

MEAN DIASTOLIC BLOOD PRESSURE (mm Hg) ± S.E.M.				
Drug or Saline	Before	After	Mean Change	p value of the difference
(1) Normal Saline	91.0 ± 3.4	92.0 ± 3.2	+ 1.0 ± 2.0	> 0.5
(2) *dl*-propranolol 0.1 mg/kg	92.6 ± 2.9	102.7 ± 3.3	+10.1 ± 1.8	< 0.001
(3) *dl*-propranolol 1.0 mg/kg	91.0 ± 3.1	97.4 ± 3.8	+ 6.4 ± 1.5	< 0.01
(4) MJ-1999 0.2 mg/kg	83.4 ± 2.6	89.4 ± 2.8	+ 6.0 ± 1.6	< 0.001
(5) MJ-1999 3.2 mg/kg	86.3 ± 3.0	92.7 ± 5.0	+ 6.3 ± 4.5	> 0.1
(6) *d*-propranolol 0.0723 mg/kg	95.5 ± 4.5	95.8 ± 3.9	+ 0.3 ± 2.2	> 0.5
(7) MJ-1999 0.2 mg/kg + *d*-propranolol 0.0723 mg/kg	95.8 ± 4.2	106.2 ± 5.7	+10.3 ± 6.6	> 0.1
(8) AY-21,011 1.4 mg/kg	89.5 ± 3.4	97.1 ± 3.3	+ 7.6 ± 3.3	< 0.05

Figure 5

Time-course of the mean systolic and diastolic blood pressure in various groups.

C = Base line value, O = coronary occlusion, C to O = effect of normal saline or drug.

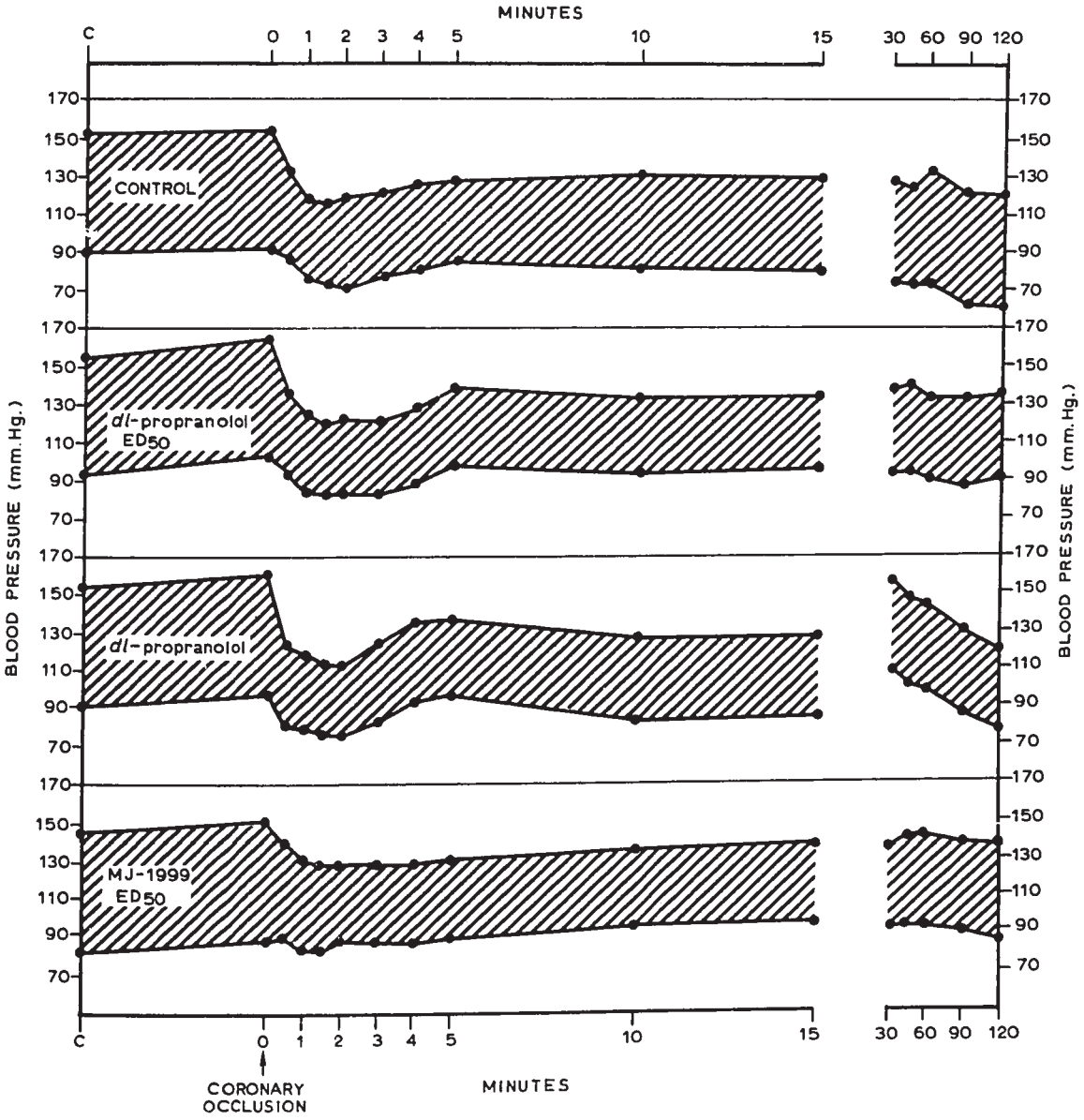
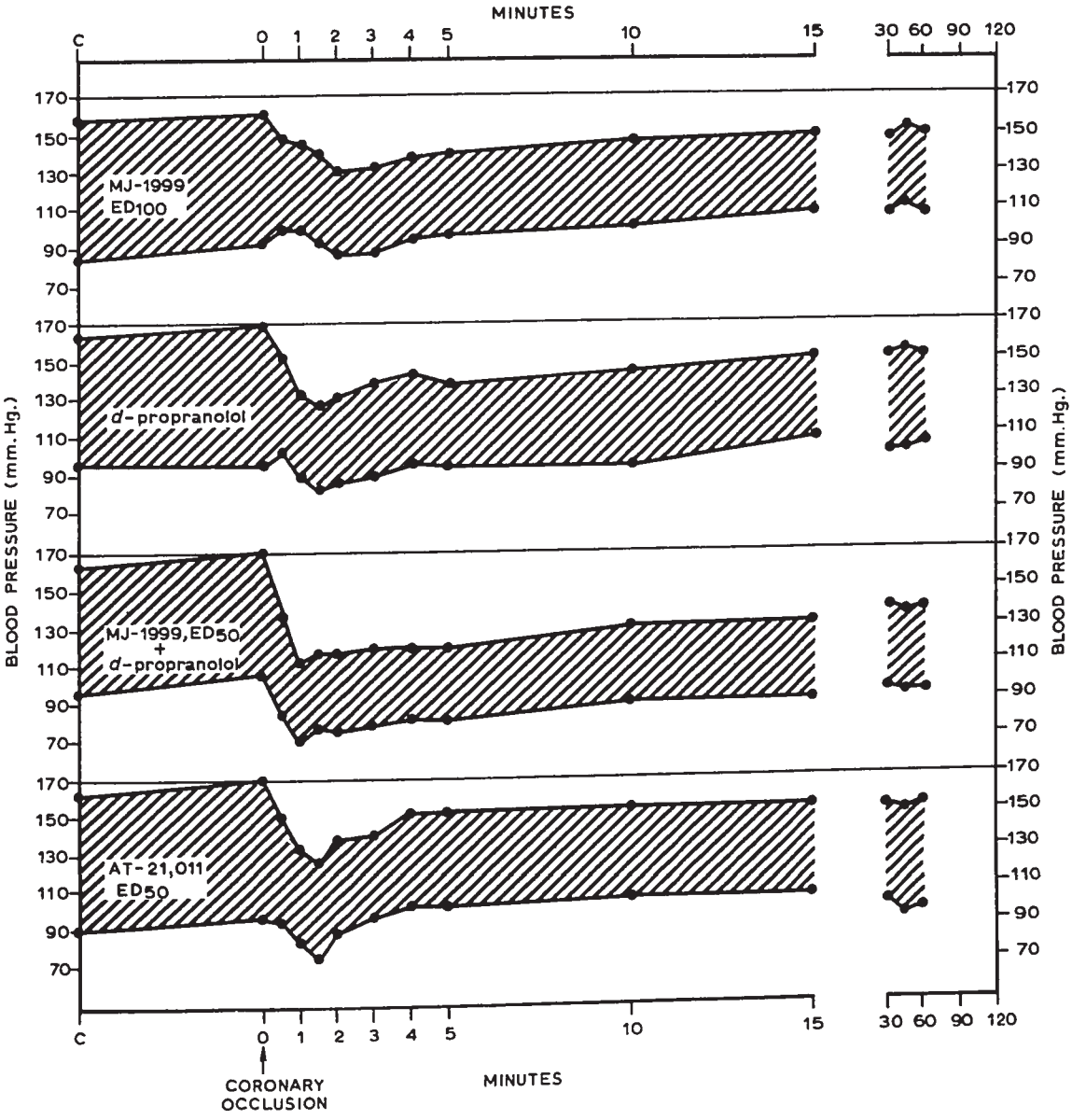

MINUTES
C
0 1 2 3 4 5
10
15
30 60 90 120
170
150
130
110
90
70
CONTROL
dl-propranolol ED50
dl-propranolol
MJ-1999 ED50
BLOOD PRESSURE (mm.Hg.)
CORONARY OCCLUSION
MINUTES

Figure 6

Time-course of the mean systolic and diastolic blood pressure in various groups.

C = Base line value, O = coronary occlusion, C to O = effect of normal saline or drug.


MINUTES
C
0 1 2 3 4 5
10
15
30 60 90 120
MJ-1999
ED100
d-propranolol
MJ-1999, ED50
+
d-propranolol
AT-21,011
ED50
BLOOD PRESSURE (mm.Hg.)
170
150
130
110
90
70
CORONARY
OCCLUSION
MINUTES

heart rate, there was a consistent pattern of a rise in blood pressure with treatment so that when changes induced by normal saline were compared with the changes caused by all the treatments, taken as a group, the difference was significant ($p < 0.05$).

D. Electrocardiogram.

Besides the change in heart rate, the only other discernable change in the electrocardiogram caused by these drugs was noted in the PR interval (Table VI). Analysis of paired data demonstrated that this interval was significantly prolonged by both dose levels of *dl*-propranolol and MJ-1999, by *d*-propranolol alone and by *d*-propranolol in combination with the ED_{50} dose of MJ-1999. The changes in the PR interval caused by normal saline and by AY-21,011 were not statistically significant. Overall analysis of variance using the changes showed that the changes in this parameter were significantly different between the groups ($p < 0.01$).

No appreciable change was noted in P waves, QRS complex, ST segment and T waves following the administration of these drugs.

IV. The effect of sudden coronary occlusion in conscious dogs.

A. General Observations.

At the time of sudden coronary occlusion resulting from traction on the exteriorized ends of the coronary ligature, most dogs struggled, some whined and a few remained quiet. Following

TABLE VI

The effect of various drugs compared to normal saline on the mean PR-interval in conscious dogs. The p values shown were determined by student's t-test using paired data. The difference of the changes between the eight groups is significant ($p < 0.01$).

The effect of various drugs compared to normal saline on the mean PR-interval in conscious dogs.

MEAN PR-INTERVAL (m.sec) ± S.E.M.				
Drug or Saline	Before	After	Mean Change	p value of the difference
(1) Normal Saline	94.7 ± 2.5	97.8 ± 3.0	+ 2.9 ± 1.7	> 0.05
(2) *dl*-propranolol 0.1 mg/kg	110.0 ± 4.4	121.3 ± 4.4	+11.3 ± 1.4	< 0.001
(3) *dl*-propranolol 1.0 mg/kg	102.5 ± 3.0	113.9 ± 3.6	+11.4 ± 1.7	< 0.001
(4) MJ-1999 0.2 mg/kg	95.4 ± 2.8	105.4 ± 3.4	+10.0 ± 1.4	< 0.001
(5) MJ-1999 3.2 mg/kg	110.6 ± 7.1	121.5 ± 6.1	+10.9 ± 2.7	< 0.01
(6) *d*-propranolol 0.0723 mg/kg	102.4 ± 5.5	105.7 ± 5.9	+ 3.3 ± 1.6	< 0.05
(7) MJ-1999 0.2 mg/kg + *d*-propranolol 0.0723 mg/kg	95.7 ± 3.8	106.5 ± 4.6	+10.7 ± 1.8	< 0.001
(8) AY-21,011 1.4 mg/kg	102.4 ± 6.1	103.9 ± 5.4	+ 1.5 ± 1.7	> 0.3

this initial response, most dogs were quiet and could easily be managed. Confinement in one position made many dogs restless after about an hour following coronary occlusion. The animals which died from ventricular fibrillation soon after coronary occlusion usually sruggled, whined and terminally had incontinence of bladder and bowel. Ventricular fibrillation was associated with instantaneous drop in blood pressure. The drop in blood pressure was gradual when the animal died with cardiac arrest following increasing degrees of atrio-ventricular block and decreasing rate of idioventricular rhythm.

B. Heart Rate.

There was an increase in heart rate in all groups immediately following the coronary occlusion reaching a peak between half a minute and two minutes and then gradually coming back towards the pre-occlusion level (Figure 4). Analysis of paired data showed that the peak increase in heart rate was significant when compared with the pre-occlusion heart rate (Table VII). The magnitude of the increases in heart rate in the various groups was not significantly different ($p > 0.05$). When the change in heart rate in the control group was compared with the change in heart rate in all the treatment groups, taken together, the difference was also not significant. The time-course of the mean heart rate for a period of one to two hours showed that it remained slightly higher than the pre-occlusion level in all groups except the control group where it returned to the pre-occlusion level within 30 minutes (Figure 4, Appendix II).

TABLE VII

Immediate effect of sudden coronary occlusion on the mean heart rate in conscious dogs. The p values shown were determined by student's t-test using non-paired data. The difference of the changes between the eight groups by overall analysis of variance is not significant; the difference in the change in Group 1 compared with Groups 2 through 8, taken together, is also not significant. The numbers in brackets indicate the number of animals available when this comparison was made.

Immediate effect of sudden coronary occlusion on the mean heart rate in conscious dogs.

MEAN HEART RATE S.E.M.				
Drug or Saline	Before Occlusion	Highest level after occlusion	Mean Change	p value of the difference
(1) Normal Saline	123.2 ± 5.0 (25)	181.6 ± 12.9 (23)	+ 58.4 ± 13.3	< 0.001
(2) *dl*-propranolol 0.1 mg/kg	99.6 ± 4.7 (25)	147.5 ± 5.9 (25)	+ 47.9 ± 6.2	< 0.001
(3) *dl*-propranolol 1.0 mg/kg	110.9 ± 5.9 (10)	141.3 ± 8.5 (10)	+ 30.4 ± 6.9	< 0.01
(4) MJ-1999 0.2 mg/kg	112.8 ± 2.9 (25)	174.6 ± 6.3 (25)	+ 61.8 ± 5.6	< 0.001
(5) MJ-1999 3.2 mg/kg	89.6 ± 4.6 (25)	128.3 ± 4.9 (25)	+ 38.7 ± 5.8	< 0.001
(6) *d*-propranolol 0.0723 mg/kg	112.3 ± 9.8 (16)	193.1 ± 24.8 (16)	+ 80.8 ± 19.6	< 0.01
(7) MJ-1999 0.2 mg/kg + *d*-propranolol 0.0723 mg/kg	111.0 ± 4.5 (12)	165.3 ± 8.0 (12)	+ 54.3 ± 8.9	< 0.001
(8) AY-21,011 1/4 mg/kg	105.8 ± 7.5 (16)	163.4 ± 15.1 (16)	+ 57.6 ± 14.3	< 0.01

C. Blood Pressure.

There was a drop in both systolic and diastolic blood pressures in all groups immediately following the coronary occlusion reaching its lowest level within two minutes and gradually coming back towards the pre-occlusion level (Figures 5 and 6). For the systolic blood pressure, the difference between the pre-occlusion level and the maximal drop following coronary occlusion, analyzed as paired data, was significant in all groups ($p < 0.02$ to 0.001) except in the group treated with *d*-propranolol where this fell just short of significance ($0.1 > p > 0.05$). However, the magnitude of the fall in the various groups was not significantly different from each other ($p > 0.05$, Table VIII).

The maximal drop in the diastolic blood pressure following coronary occlusion was statistically significant in Groups 1, 2 and 7 and was not significant in other groups (Figures 5 and 6, Table IX). As with the systolic blood pressure, the magnitude of the fall of diastolic blood pressure in the various groups was not significantly different from each other ($p > 0.05$).

As shown in Figures 5 and 6, after the initial drop, both systolic and diastolic blood pressures returned quickly towards the pre-occlusion levels, but remained lower than the pre-occlusion level in all groups during the period of observation of one to two hours (Appendix II). The drop in the systolic pressure was more than that in the diastolic pressure, so that following coronary occlusion, the pulse pressure was lower than

TABLE VIII

Immediate effect of sudden coronary occlusion on the mean systolic blood pressure in conscious dogs. The p values shown were determined by student's t-test using non-paired data. The difference of the changes between the eight groups by overall analysis of variance is not significant. The difference in the change in Group 1 compared with Groups 2 through 8, taken together, is also not significant. The numbers in brackets indicate the number of animals available when this comparison was made.

Immediate effect of sudden coronary occlusion on the mean systolic blood pressure in conscious dogs.

MEAN SYSTOLIC BLOOD PRESSURE (mm Hg) ± S.E.M.				
Drug or Saline	Before Occlusion	Lowest level after occlusion	Mean Change	p value of the difference
(1) Normal Saline	154.3 ± 4.4 (25)	118.7 ± 8.6 (23)	-36.3 ± 6.6	< 0.001
(2) *dl*-propranolol 0.1 mg/kg	164.8 ± 4.2 (25)	120.8 ± 8.1 (21)	-44.2 ± 6.1	< 0.001
(3) *dl*-propranolol 1.0 mg/kg	161.4 ± 5.9 (10)	113.8 ± 12.7 (10)	-47.6 ± 9.9	< 0.01
(4) MJ-1999 0.2 mg/kg	151.8 ± 4.0 (25)	128.6 ± 5.1 (20)	-23.2 ± 5.3	< 0.001
(5) MJ-1999 3.2 mg/kg	161.0 ± 7.7 (12)	131.3 ± 8.4 (11)	-29.7 ± 4.8	< 0.02
(6) *d*-propranolol 0.0723 mg/kg	168.5 ± 8.4 (16)	126.7 ± 7.0 (9)	-31.8 ± 14.3	> 0.05
(7) MJ-1999 0.2 mg/kg + *d*-propranolol 0.0723 mg/kg	170.0 ± 7.1 (12)	112.4 ± 6.8 (11)	-57.6 ± 8.0	< 0.001
(8) AY-21,011 1.4 mg/kg	168.6 ± 5.9 (16)	125.7 ± 13.8 (14)	-42.9 ± 13.9	< 0.01

TABLE IX

Immediate effect of sudden coronary occlusion on the mean diastolic blood pressure in conscious dogs. The p values shown were determined by student's t-test using non-paired data. The difference of the changes between the eight groups by overall analysis of variance is not significant; the difference in the change in Group 1 compared with Groups 2 through 8, taken together, is also not significant. The numbers in brackets indicate the number of animals available when this comparison was made.

Immediate effect of sudden coronary occlusion on the mean diastolic blood pressure in conscious dogs

MEAN DIASTOLIG BLOOD PRESSURE (mm Hg) ± S.E.M.				
Drug or Saline	Before Occlusion	Lowest level after occlusion	Mean Change	p value of the difference
(1) Normal Saline	92.0 ± 3.3 (25)	71.5 ± 6.1 (11)	-20.5 ± 3.4	< 0.01
(2) *dl*-propranolol 0.1 mg/kg	102.7 ± 3.4 (25)	83.0 ± 7.4 (20)	-19.7 ± 6.0	< 0.02
(3) *dl*-propranolol 1.0 mg/kg	97.4 ± 4.0 (10)	75.8 ± 10.7 (10)	-21.6 ± 9.4	> 0.05
(4) MJ-1999 0.2 mg/kg	89.4 ± 2.9 (25)	83.8 ± 5.3 (24)	- 5.6 ± 5.2	> 0.3
(5) MJ-1999 3.2 mg/kg	92.7 ± 5.2	86.9 ± 7.5	- 5.8 ± 5.6	> 0.3
(6) *d*-propranolol 0.0723 mg/kg	95.8 ± 4.0 (16)	82.7 ± 10.6 (9)	-13.1 ± 12.2	> 0.2
(7) MJ-1999 0.2 mg/kg + *d*-propranolol 0.0723 mg/kg	106.2 ± 5.9 (12)	70.2 ± 6.7 (11)	-36.0 ± 6.7	< 0.001
(8) AY-21,011 1.4 mg/kg	97.1 ± 3.4 (16)	81.8 ± 9.6 (14)	- 5.3 ± 10.4	> 0.1

that before the occlusion.

V. Mortality rate following sudden occlusion of the circumflex branch of the left coronary artery in conscious dogs.

The two-hour and 24-hour mortality rates following sudden coronary occlusion in the eight experimental groups are summarized in Table X and Figure 7. The cumulative fatality rates, plotted against time, are shown in Figure 8. The difference in the mortality rates between the eight groups is highly significant ($p < 0.001$ by overall X^2- test). The mortality rates of Groups 2, 4, 5, 6 and 8 are significantly lower than that of the control group, whereas the mortality rates of Groups 3 and 6 are not. As far as the mortality rate is concerned, there, then appear to be two distinct categories - one with high mortality (Groups 1, 3 and 6) and one with low mortality (Groups 2, 4, 5, 7 and 8). The difference in the mortality rate between the various groups in each of these two categories is not statistically significant ($p > 0.75$).

Group 1 (Control group):

Eighteen out of 25 (72 percent) dogs in the control group died between 45 seconds and 14 minutes following coronary occlusion. It should be noted that most deaths in this group occurred within the first five minutes of coronary occlusion, there being only two dogs dying after 5.25 minutes.

Ventricular fibrillation was the cause of early deaths in every animal in this group. Ventricular fibrillation was usually

Table X

The mortality rate following sudden occlusion of the circumflex branch of the left coronary artery in the conscious dog. The difference in the mortality rates between the various groups is highly significant ($p < 0.001$ by χ^2-test).

EXPERIMENTAL CORONARY OCCLUSION

GROUP	NUMBER OF DOGS	TWO-HOUR MORTALITY		TWENTY-FOUR-HOUR MORTALITY		SIGNIFICANCE OF THE DIFFERENCE*
		NUMBER	PERCENT	NUMBER	PERCENT	CONTROL vs TREATMENT
CONTROL	25	18	72	21	84	
PROPRANOLOL 0.1 mg/kg	25	6	24	7	28	$p < 0.001$
PROPRANOLOL 1.0 mg/kg	10	7	70	8	80	$p > 0.750$
MJ – 1999 0.2 mg/kg	25	7	28	10	40	$p < 0.005$
MJ – 1999 3.2 mg/kg	12	2	16.7	2	16.7	$p < 0.005$
d-PROPANOLOL 0.0723 mg/kg	16	11	68.8	11	68.8	$p > 0.100$
MJ-1999 0.2 mg/kg + *d*-PROPRANOLOL 0.0723 mg/kg	12	2	16.7	2	16.7	$p < 0.005$
AY-21,011 1.4 mg/kg	16	5	31.3	6	37.5	$p < 0.025$

* OVERALL $p < 0.001$ BY X^2-TEST

Figure 7

Mortality rate following sudden coronary occlusion in conscious dogs.

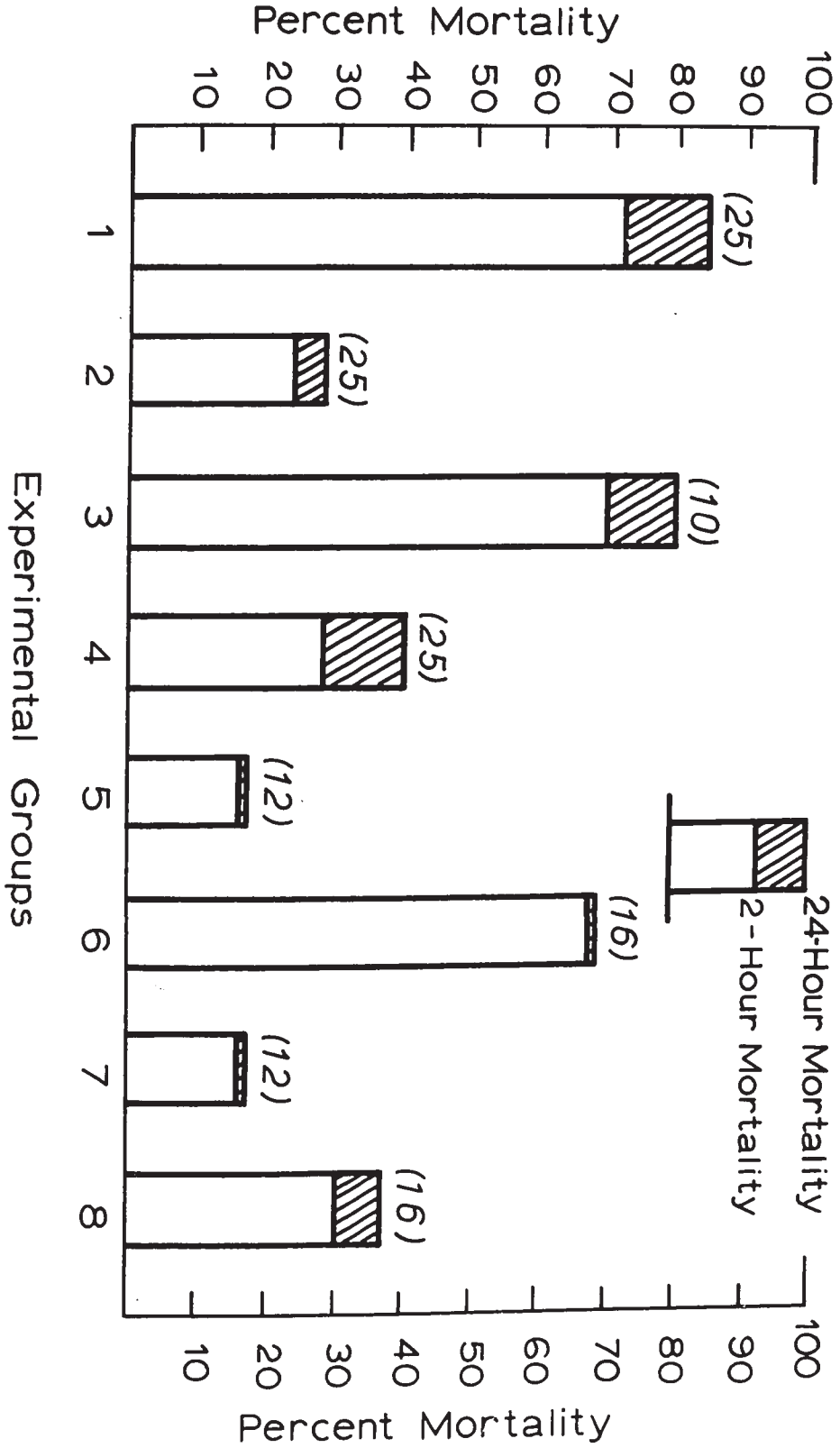
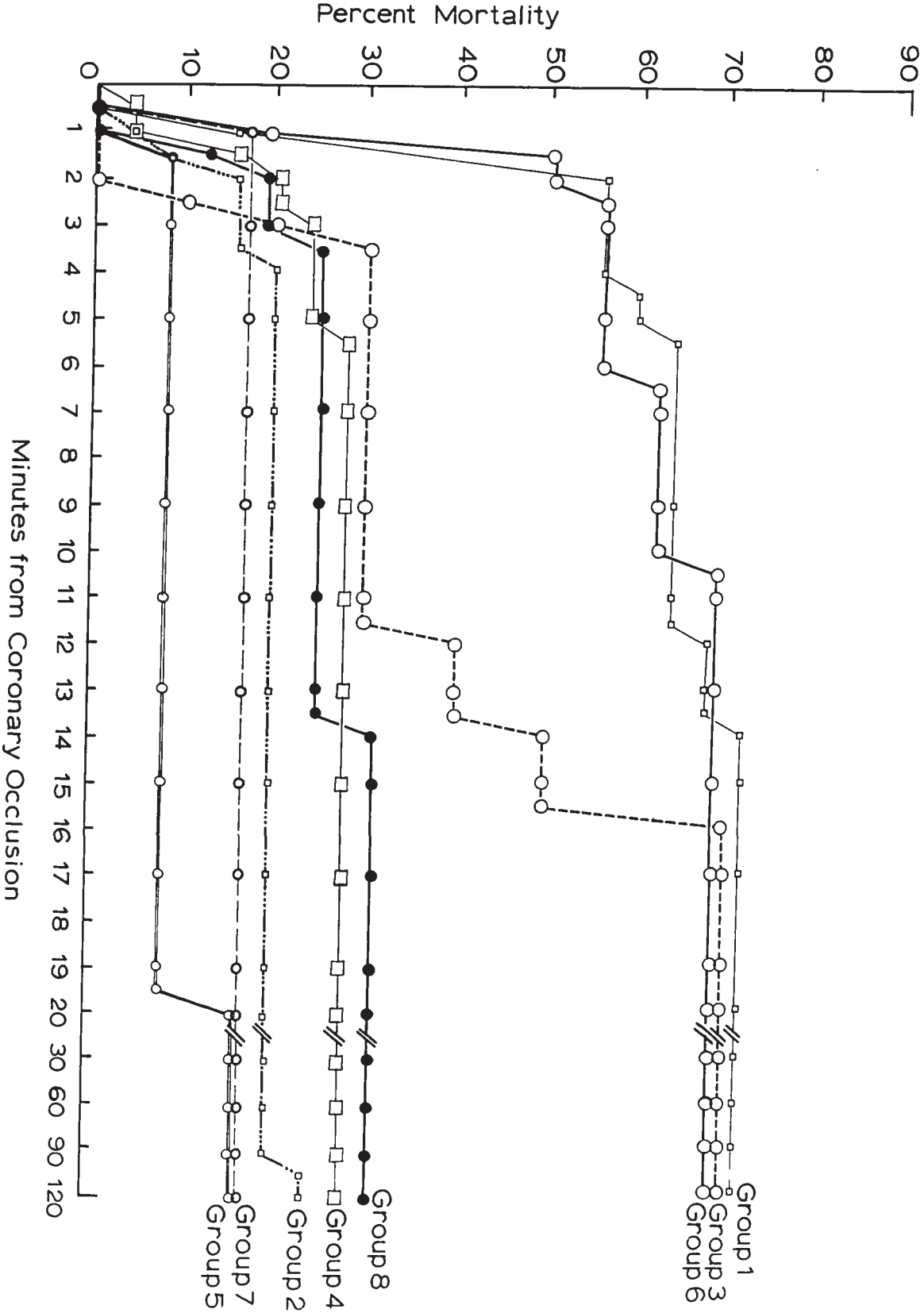

Percent Mortality
10
20
30
40
50
60
70
80
90
100
(25)
(25)
(10)
(25)
(12)
(16)
(12)
(16)
1
2
3
4
5
6
7
8
Experimental Groups
24-Hour Mortality
2-Hour Mortality
Percent Mortality

Figure 8

Cumulative fatality rate in various groups following coronary occlusion.


Percent Mortality
0
10
20
30
40
50
60
70
80
90
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 30 60 90 120
Minutes from Coronary Occlusion
Group 1
Group 3
Group 6
Group 8
Group 4
Group 2
Group 7
Group 5

preceded by frequent ventricular ectopic beats followed by ventricular tachycardia. Figure 9 illustrates an experiment showing the terminal events in electrocardiogram and blood pressure in an animal which succumbed to coronary occlusion with ventricular fibrillation as the mechanism of death.

Three additional animals in this group died between two and 24 hours following coronary occlusion, bringing the 24-hour death rate to 84 percent. The exact mechanism of these delayed deaths was unknown.

Group 2 (*dl*-propranolol, 0.1 mg/kg):

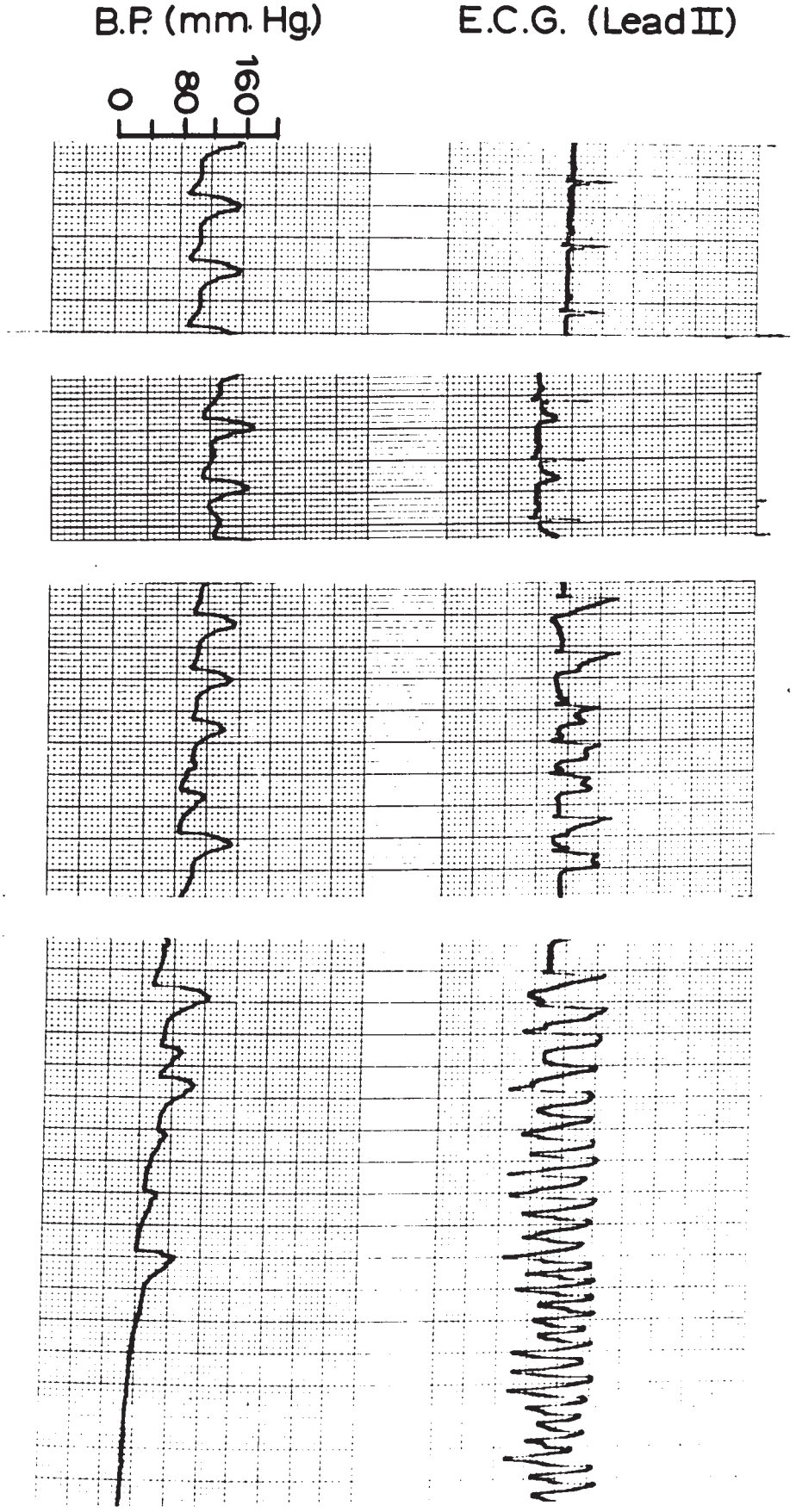
Only six out of 25 (24 percent) dogs in this group died during the two-hour observation period. Five of these deaths took place within the first five minutes of the coronary occlusion, ending in ventricular fibrillation. The sixth death of this group occurred at 100 minutes. This last dog had complete atrio-ventricular block at 55 minutes and died with cardiac standstill after sustaining several episodes of Stokes-Adams attacks. Figure 10 shows the electrocardiographic and blood pressure changes in one animal of this group surviving the coronary occlusion.

One additional dog died between two and 24 hours after coronary occlusion, bringing the 24-hour death rate to 28 percent for this group. The exact mechanism of this delayed death is not known.

Figure 9

Electrocardiographic and blood pressure changes in a control dog dying from ventricular fibrillation.

A. Pre-occlusion.
B. Fifteen seconds post-occlusion.
C. One minute post-occlusion.
D. One minute, 10 seconds post-occlusion.


B.P. (mm. Hg)
E.C.G. (Lead II)
0
80
160
A
B
C
D

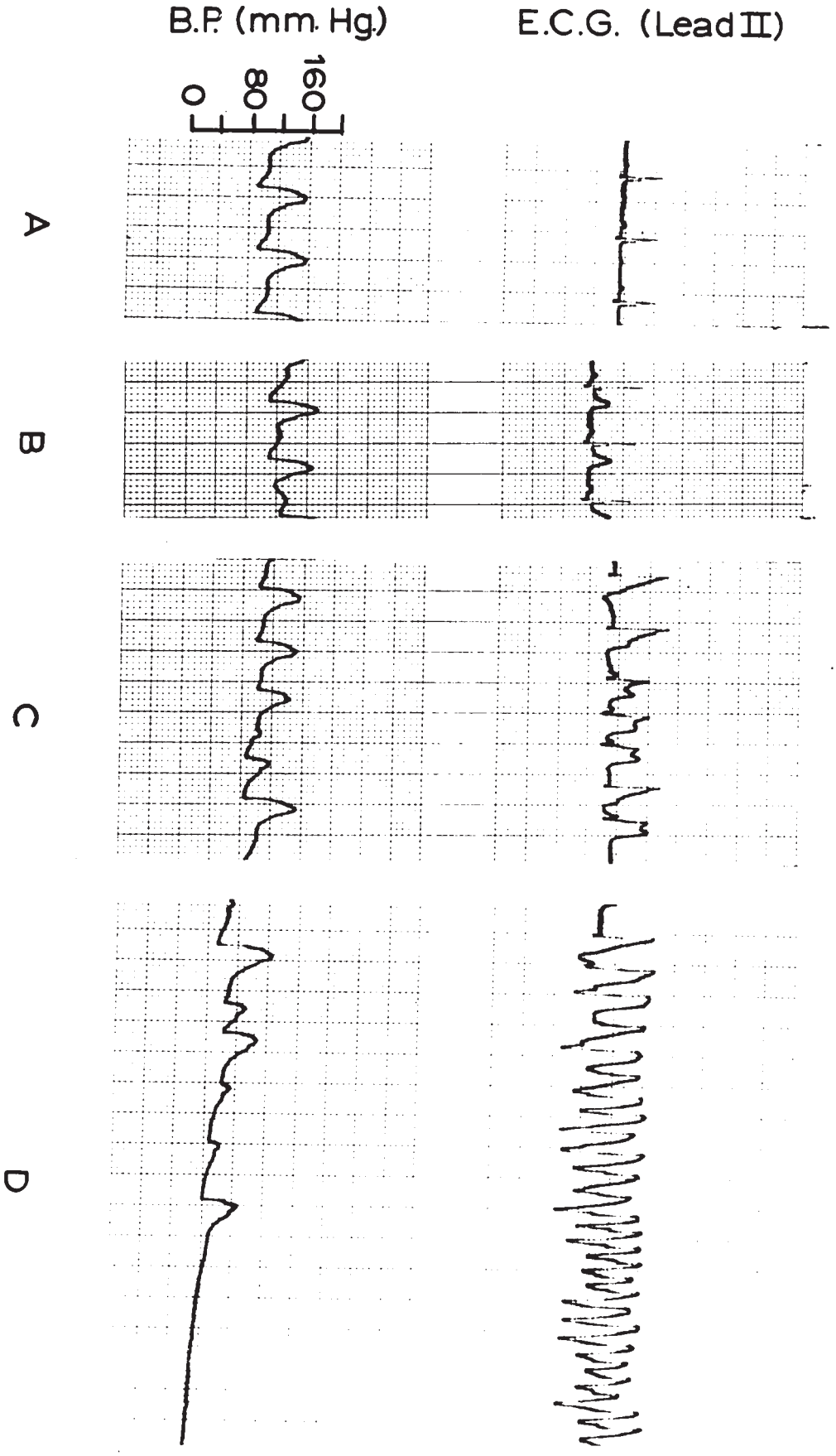
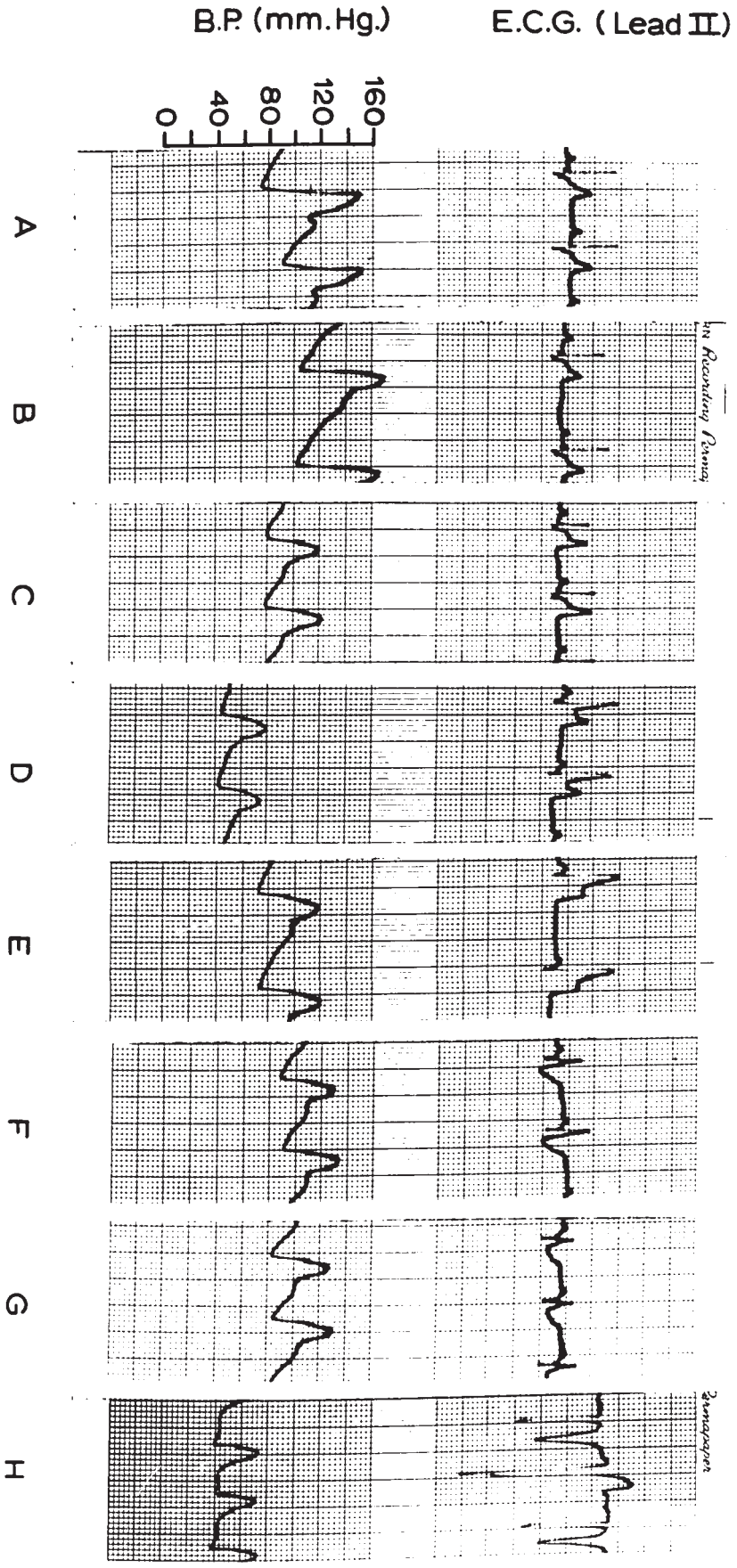
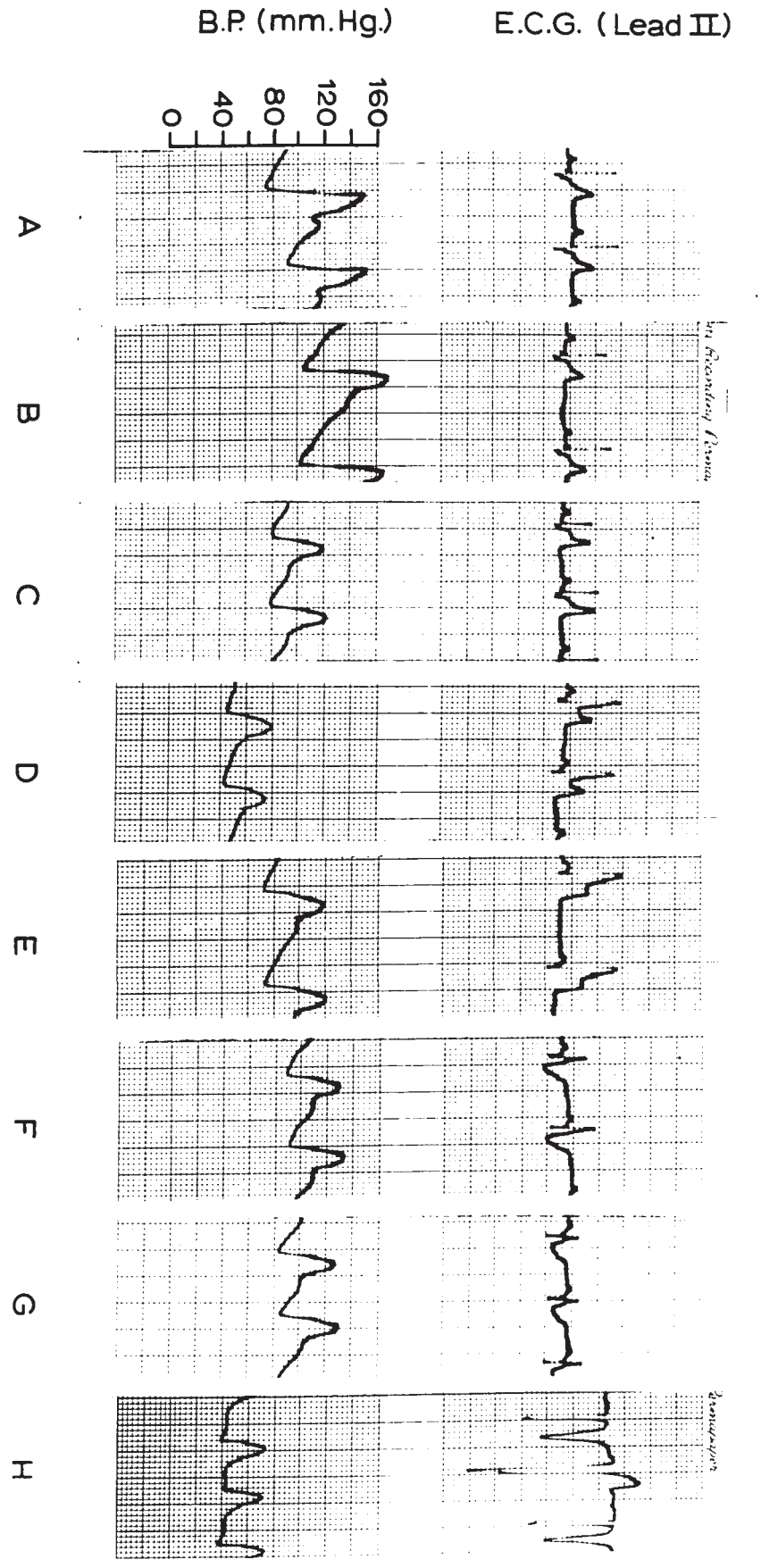

B.P. (mm. Hg.)
E.C.G. (Lead II)
0
80
160
A
B
C
D

Figure 10

Electrocardiographic and blood pressure changes in a Group 2 dog surviving acute coronary occlusion.

A. Pre-occlusion (control).
B. Five minutes after *dl*-propranolol (0.1 mg/kg)
C. One minute post-occlusion.
D. Two minutes post-occlusion.
E. Ten minutes post-occlusion.
F. One hour post-occlusion.
G. Two hours post-occlusion.
H. Twenty-four hours post-occlusion.


B.P. (mm.Hg.)
E.C.G. (Lead II)
0
40
80
120
160
A
B
C
D
E
F
G
H
Ink Recording Pen
Armature


B.P. (mm.Hg.)
E.C.G. (Lead II)
0
40
80
120
160
A
B
C
D
E
F
G
H

Group 3 (*dl*-propranolol, 1.0 mg/kg):

There were seven (70 percent) early deaths out of 10 animals in this group. Three of the seven early deaths were due to cardiac standstill, the remaining four being due to ventricular fibrillation. The time of death due to ventricular fibrillation in the animals of this group was somewhat delayed. While in the control group most of the ventricular fibrillation occurred within the first five minutes, three out of four ventricular fibrillations in this group occurred well beyond this time following coronary occlusion (Figure 8). Figure 11 illustrates the electrocardiographic and blood pressure changes in a dog of this group dying from cardiac standstill.

There was one more death occurring between two and 24 hours. Thus the 24-hour mortality rate in this group was 80 percent.

Group 4 (MJ-1999, 0.2 mg/kg):

Seven out of 25 dogs (28 percent) of this group died between 30 seconds and five minutes 20 seconds after coronary occlusion. Ventricular fibrillation was the mechanism of death in all seven deaths of this group. Three more dogs died between two and 24 hours, the 24-hour death rate being 40 percent. The exact cause of these three delayed deaths, as in other groups, was not known.

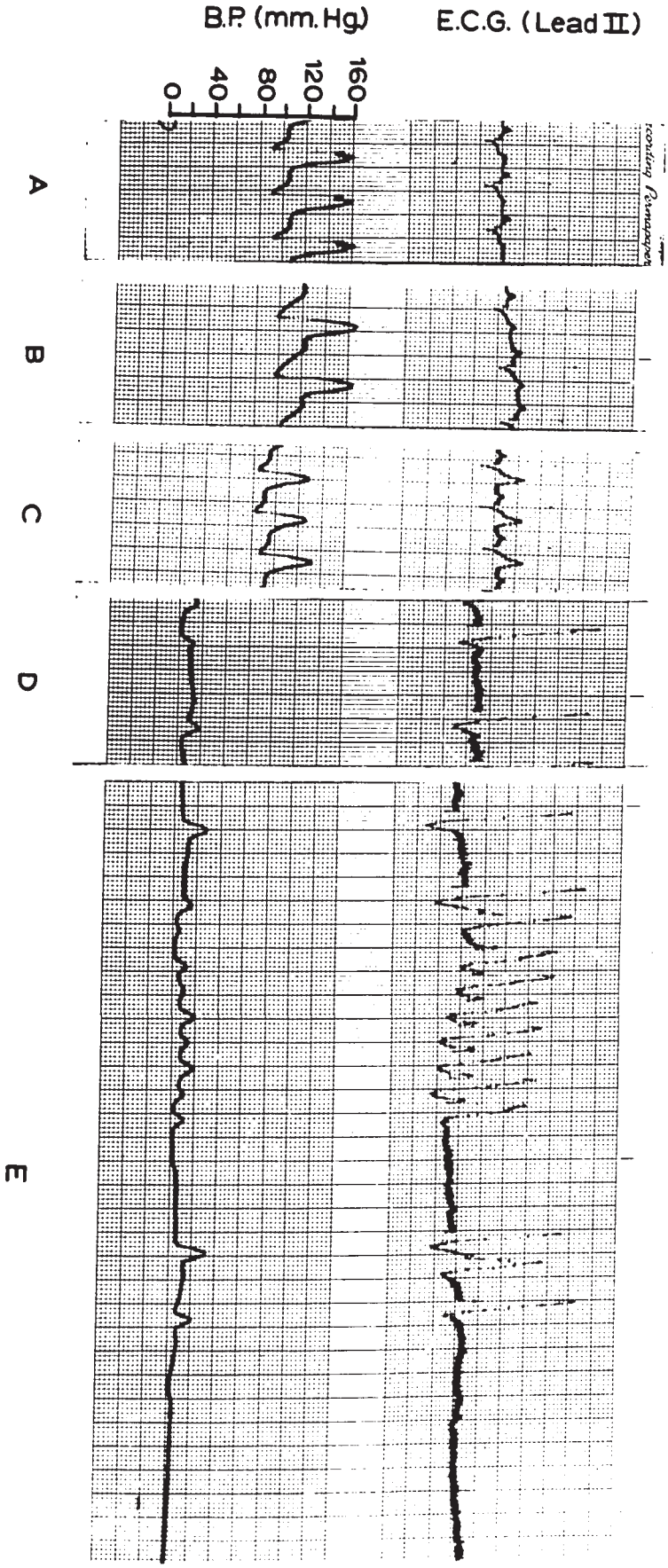
Group 5 (MJ-1999, 3.2 mg/kg):

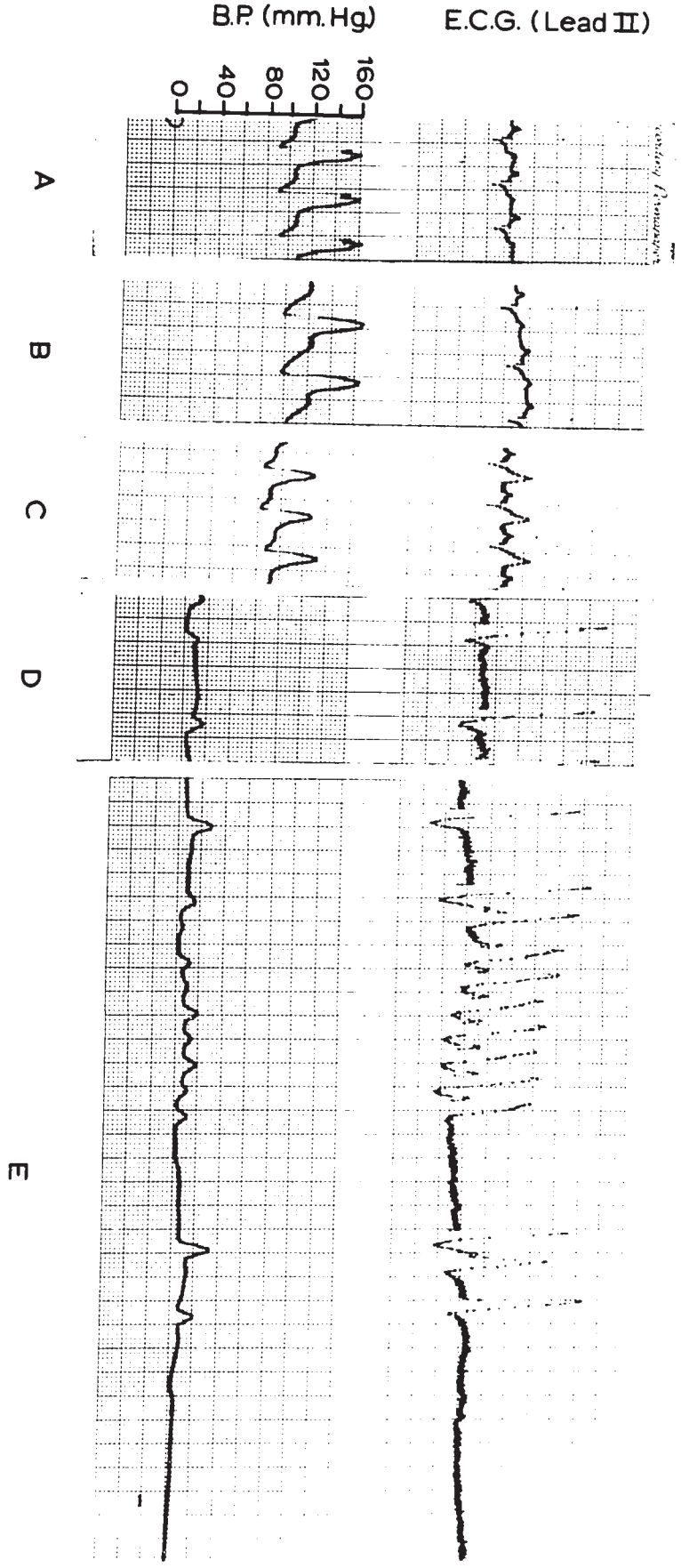
There were only two (16.7 percent) early deaths out of 12 animals in this group. The deaths occurred one minute 48 seconds

Figure 11

Electrocardiographic and blood pressure changes in a Group 3 dog dying from cardiac standstill.

A. Control electrocardiogram.
B. Five minutes after *dl*-propranolol (1.0 mg/kg)
C. Thirty seconds after coronary occlusion
D. Two minutes, 30 seconds after coronary occlusion.
E. Cardiac standstill 3 minutes and 10 seconds after coronary occlusion.


B.P. (mm. Hg)
E.C.G. (Lead II)
0
40
80
120
160
A
B
C
D
E


B.P. (mm.Hg)
E.C.G. (Lead II)
0
40
80
120
160
A
B
C
D
E

and 20 minutes after coronary occlusion. The mechanism of death in each case was ventricular fibrillation. There were no additional delayed deaths in this group.

Group 6 (*d*-propranolol, 0.0723 mg/kg):

Eleven out of 16 (68.8 percent) dogs in this group died between 40 seconds and 10 minutes 30 seconds after coronary occlusion. All these deaths were due to ventricular fibrillation. There were no further 24-hour deaths in this group.

Group 7 (MJ-1999, 0.2 mg/kg + *d*-propranolol, 0.0723 mg/kg):

Only two out of 12 dogs (16.7 percent) of this group died soon after coronary occlusion. Both deaths were due to ventricular fibrillation and were at 50 and 61 seconds following coronary occlusion. There were no delayed deaths in this group.

Group 8 (AY-21,011, 1.4 mg/kg):

Five out of 16 (31.3 percent) dogs of this group died between one minute 12 seconds and 14 minutes following coronary occlusion. Three of the early deaths were due to ventricular fibrillation, the remaining two being due to cardiac standstill. There was an additional death occurring between two and 24 hours after coronary occlusion. The mechanism of this delayed death was not known.

VI. Electrocardiographic Features

A. General Observations.

Thoracotomy, which was performed for the preparation of the dogs for coronary occlusion, caused no major change in the electrocardiogram.

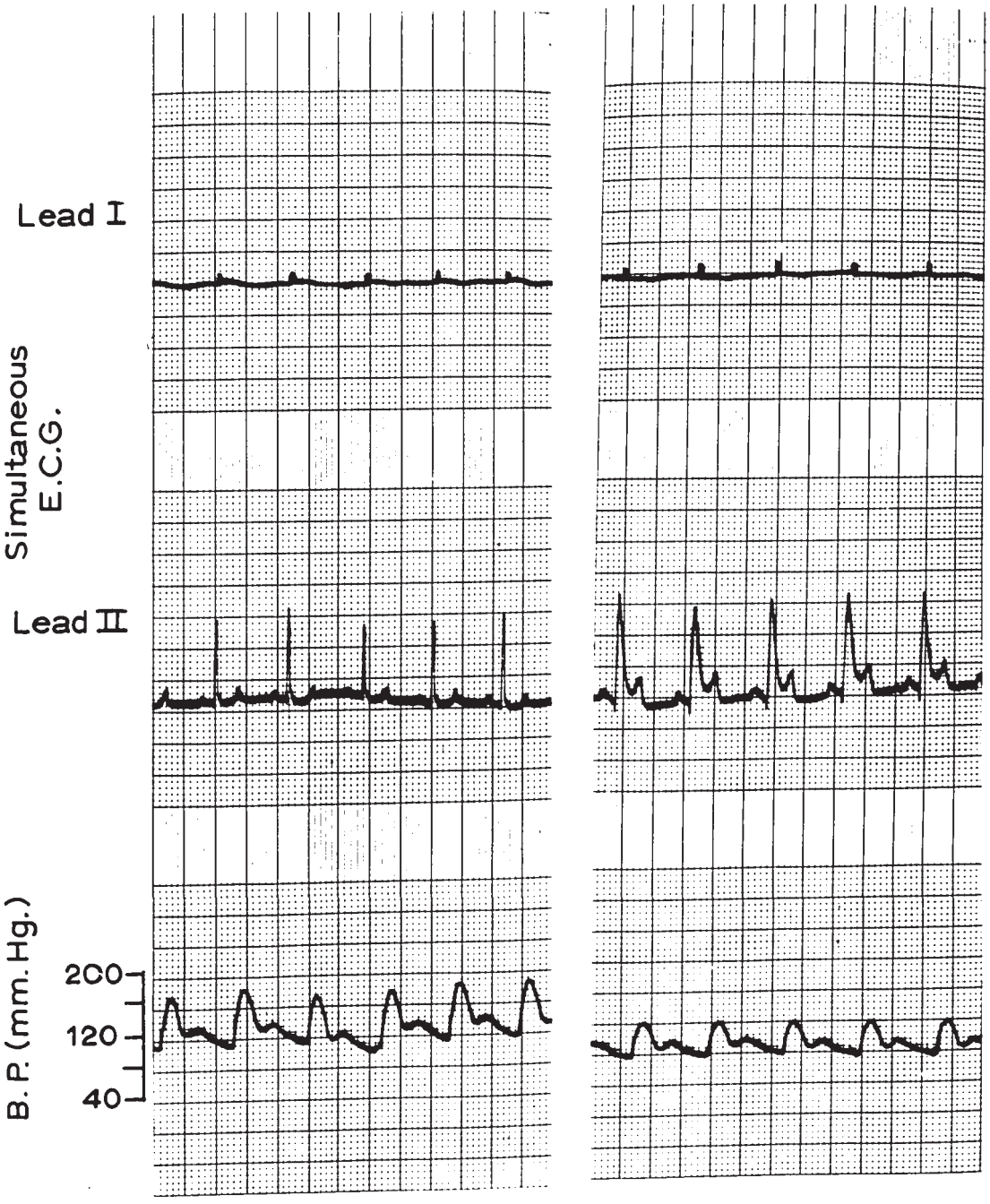
The sequence of electrocardiographic changes following sudden occlusion of the circumflex branch of the left coronary artery is shown in Figure 10. Within seconds after the coronary occlusion, the T wave, when inverted, became upright, tall and peaked in leads II, III and aVF. Soon there was progressive elevation of the ST segment. This high takeoff of the ST segment distorted the QRS complex giving the latter an appearance of having severe intraventricular conduction defect. Simultaneous recording of leads I and II revealed this change to be more apparent than real (Figure 12). There was a great variability in the time of the return of the elevated ST segment to the isoelectric line. In many instances the ST segment did not return to the isoelectric line during the period of observation of one to two hours. Inversion of the first part of the T wave was noted in many cases with the return of the ST segment to the isoelectric line. The Q wave recognizably increased in magnitude in lead II as early as 30 minutes following coronary occlusion and by 120 minutes they were quite prominent, but the R wave still persisted at that time. When the electrocardiogram was repeated 24 hours later, all positive forces in leads II, III and aVF were abolished, ST segment became isoelectric, T wave deeply negative and cove-shaped

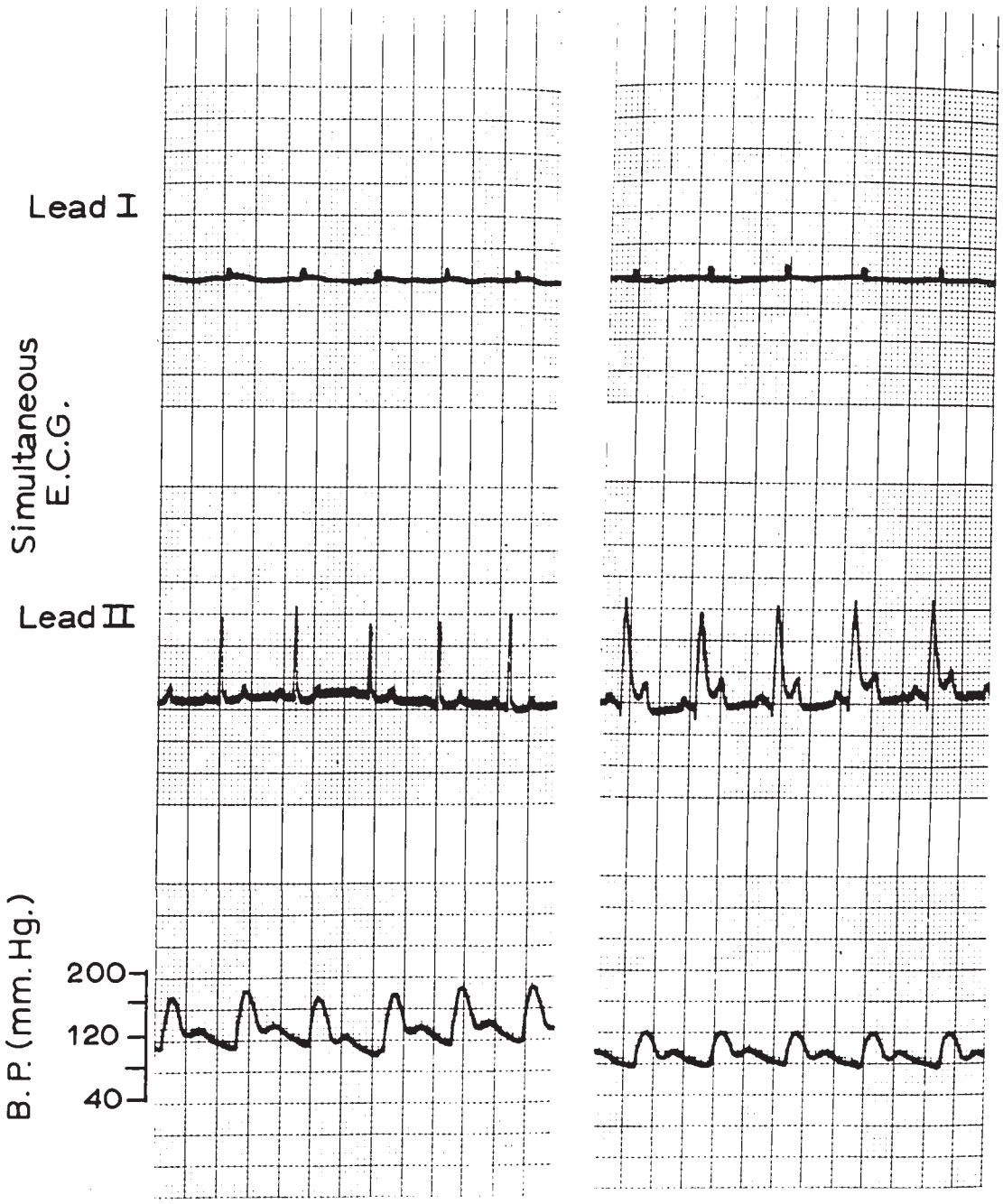
Figure 12

Simultaneous recording of two electrocardiographic leads to elucidate the apparent intraventricular conduction defect in lead II.

A. Control electrocardiogram of a Group 6 dog showing normal QRS duration in leads I and II, recorded simultaneously.

B. Electrocardiogram of the same dog 3.5 minutes after coronary occlusion showing widening of QRS complex in lead II; no widening is noted in simultaneously recorded lead I.


Lead I
Simultaneous E.C.G.
Lead II
B.P. (mm.Hg.)
200
120
40
A
B


Lead I
Simultaneous E.C.G.
Lead II
B.P. (mm. Hg.)
200
120
40
A
B

and the QRS was represented by only QS complex.

Very frequently, one or two ectopic beats were noted when the exteriorized ends of the cord around the coronary artery branch were pulled to produce coronary occlusion. These were, in all probability, produced mechanically by the pull of the cord. When present, these mechanically-induced ventricular ectopic beats were similar in configuration in different animals. The spontaneous ectopic beats following coronary occlusion were strikingly different in configuration from the mechanically-induced ectopic beats even in the same dog (Figure 13). Another frequent observation was a temporary wandering of the pacemaker indicated by changes in the size and shape of P waves and in the duration of the PR interval following coronary occlusion. This was transient, disappearing in about 5 minutes following coronary occlusion.

B. Cardiac Arrhythmias:

The fatal arrhythmias have been referred to in the section dealing with the mortality rate. Mechanically-induced ectopic beats with pulling of the cord and transient shift in the pacemaker site following coronary occlusion have been mentioned above. The non-fatal arrhythmias encountered in the present study include the following:

1. Ventricular ectopic beats.

   All ventricular ectopic beats were counted from the direct writer record as well as from re-playing the magnetic tape.

Figure 13

Mechanically-induced and spontaneous ectopic beats following coronary occlusion.

A(M) Electrocardiogram of a Group 4 dog showing mechanically-induced ectopic beats.

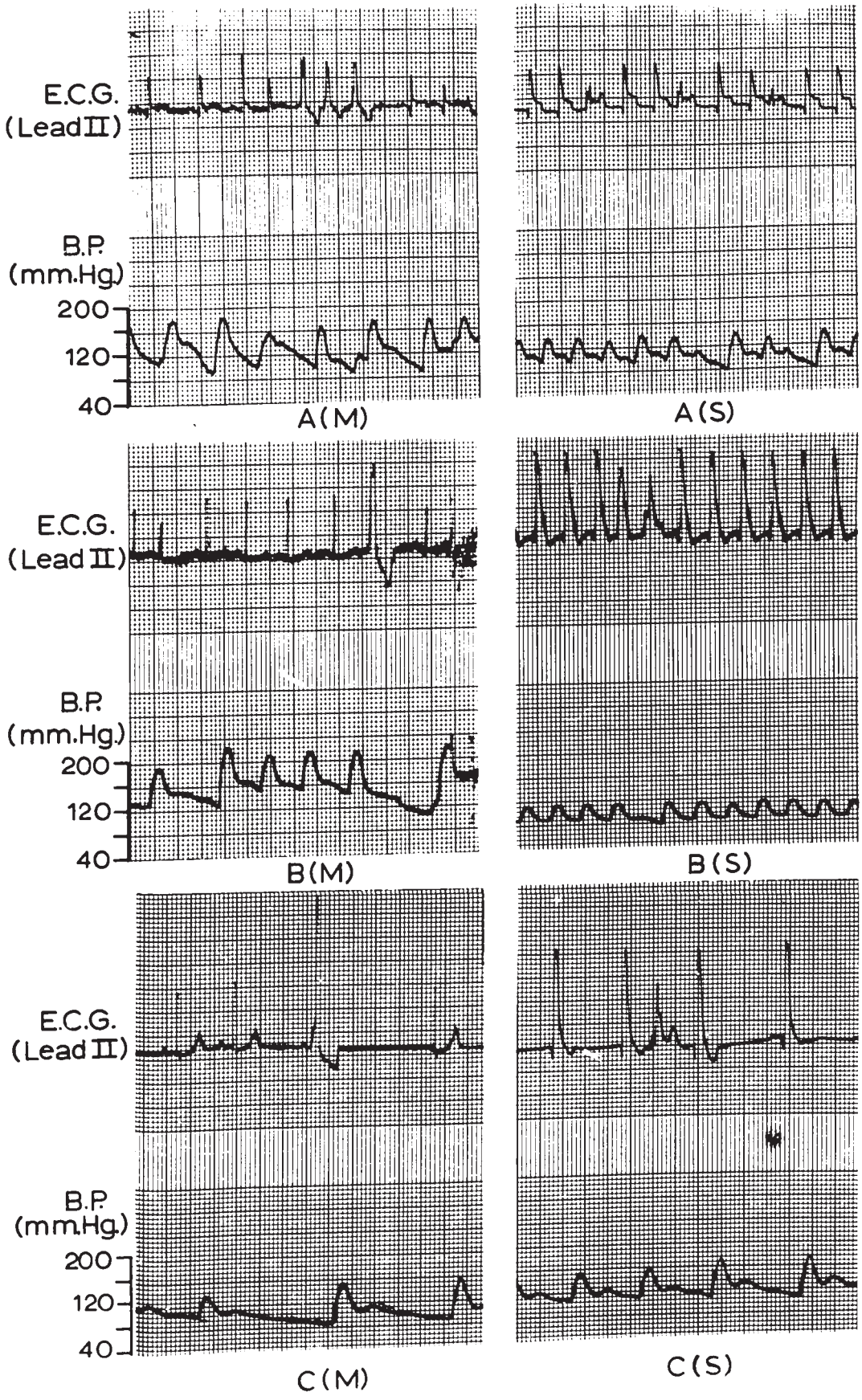
A(S) Same dog as above, two minutes after coronary occlusion showing spontaneous ectopic beats.

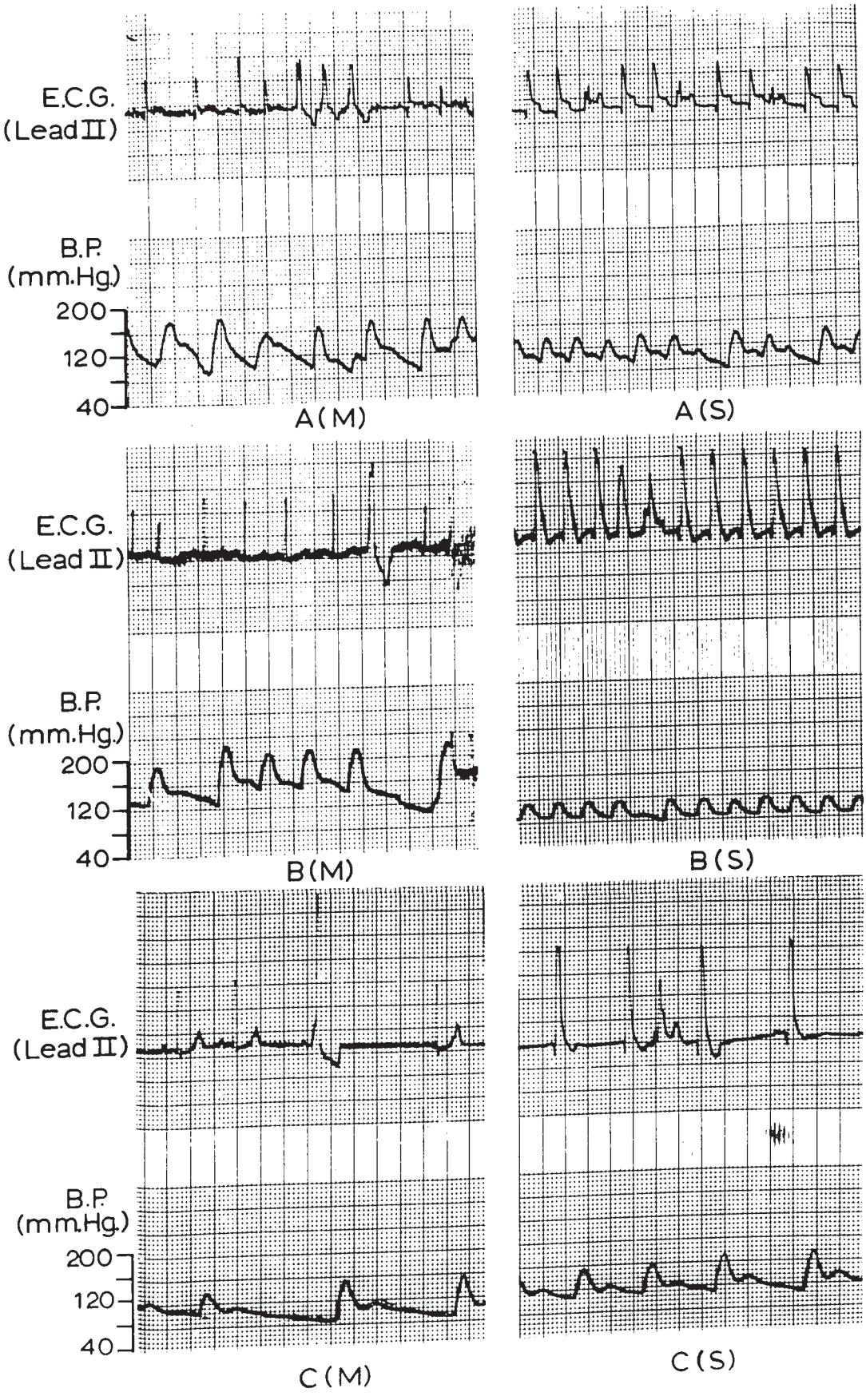
B(M) Electrocardiogram of a Group 7 dog showing mechanically-induced ectopic beats.

B(S) Same dog as above, 4 minutes after coronary occlusion showing spontaneous ectopic beats.

C(M) Electrocardiogram of a Group 4 dog showing mechanically-induced ectopic beats.

C(S) Same dog as above, 90 seconds after coronary occlusion showing spontaneous ectopic beats.


E.C.G.
(Lead II)
B.P.
(mm.Hg.)
200
120
40
A(M)
A(S)
E.C.G.
(Lead II)
B.P.
(mm.Hg.)
200
120
40
B(M)
B(S)
E.C.G.
(Lead II)
B.P.
(mm.Hg.)
200
120
40
C(M)
C(S)


E.C.G.
(Lead II)
B.P.
(mm.Hg.)
200
120
40
A(M)
A(S)
E.C.G.
(Lead II)
B.P.
(mm.Hg.)
200
120
40
B(M)
B(S)
E.C.G.
(Lead II)
B.P.
(mm.Hg.)
200
120
40
C(M)
C(S)

As shown in Figure 14, the quality of reproduction of data from the magnetic tape was satisfactory. All ventricular ectopic beats were further classified into:

(a) 'ventricular premature beats', when they appeared singly or in groups of three or less.

(b) 'ventricular tachycardia', when they occurred in groups of 4 or more.

2. First, second and third degree atrioventricular block.
3. Sinus arrest.
4. Atrial premature beats.
5. Atrial tachycardia.
6. Atrial fibrillation.
7. Ventricular fibrillation.

The following section describes the observed occurrance of each form of arrhythmia.

1. Ventricular Ectopic Beats

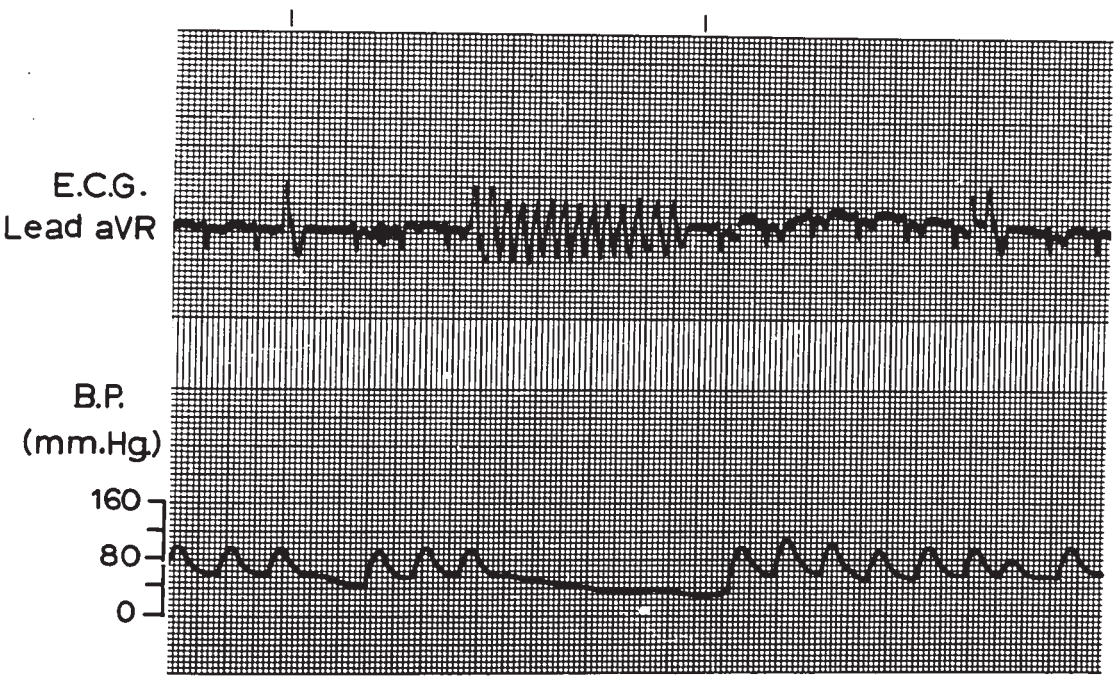
Ventricular ectopic beats, when present, usually appeared within two minutes of coronary occlusion, rapidly increased in frequency reaching peaks between 2 and 5 minutes and then gradually decreased in frequency, so that there were very few ectopic beats beyond 15 minutes of coronary occlusion (Figures 15 and 16). Non-fatal ventricular ectopic beats were encountered in 68.8 to 92 percent of cases in various groups (Table XI). The difference in the incidence of 'all ventricular ectopic beats' in the various groups is

Figure 14

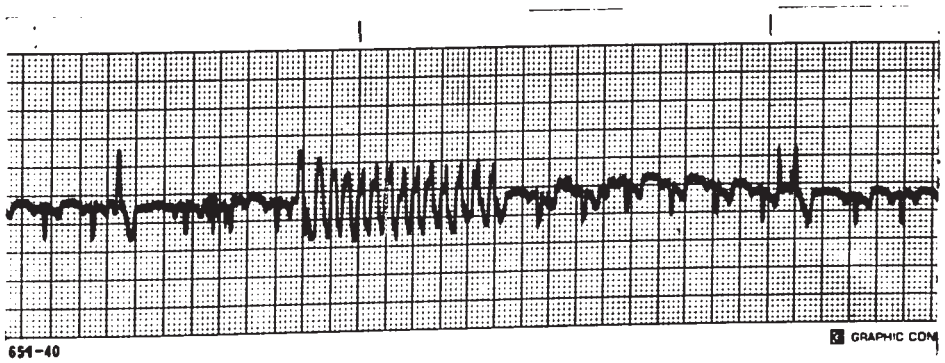
Quality of reproduction of data from the magnetic tape.

A. Direct recording of electrocardiogram of a Group 6 dog 6.5 minutes after coronary occlusion.

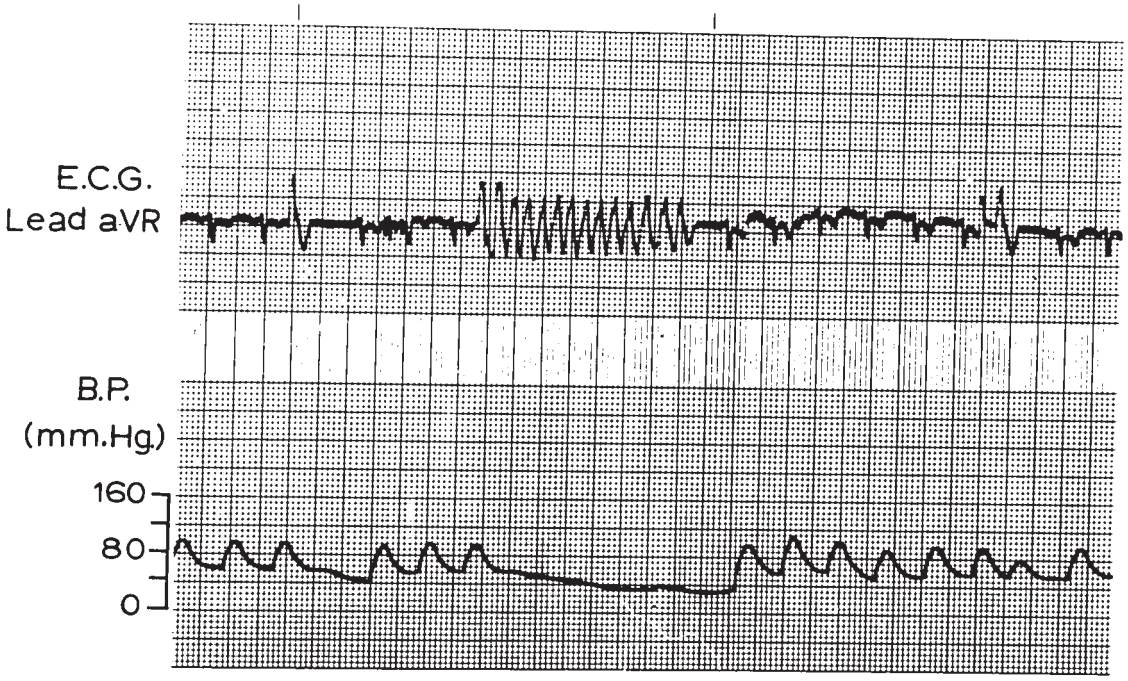
B. Reproduction of the same segment of electrocardiogram from the magnetic tape.



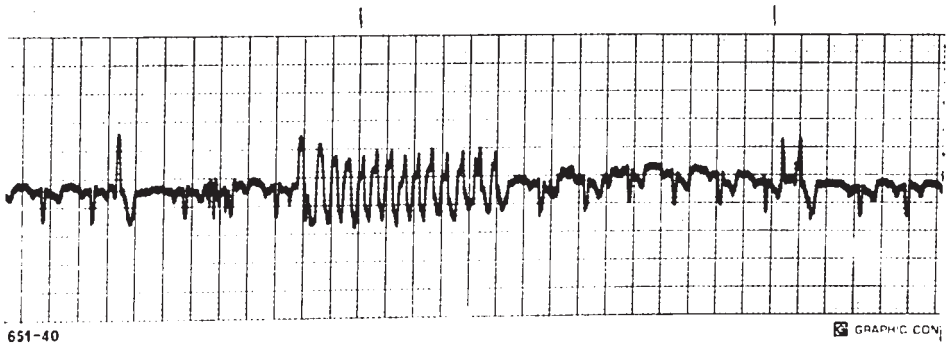
A



B



A



B

Figure 15

Ventricular ectopic beats following sudden coronary occlusion in Groups 1, 2, 3 and 4.

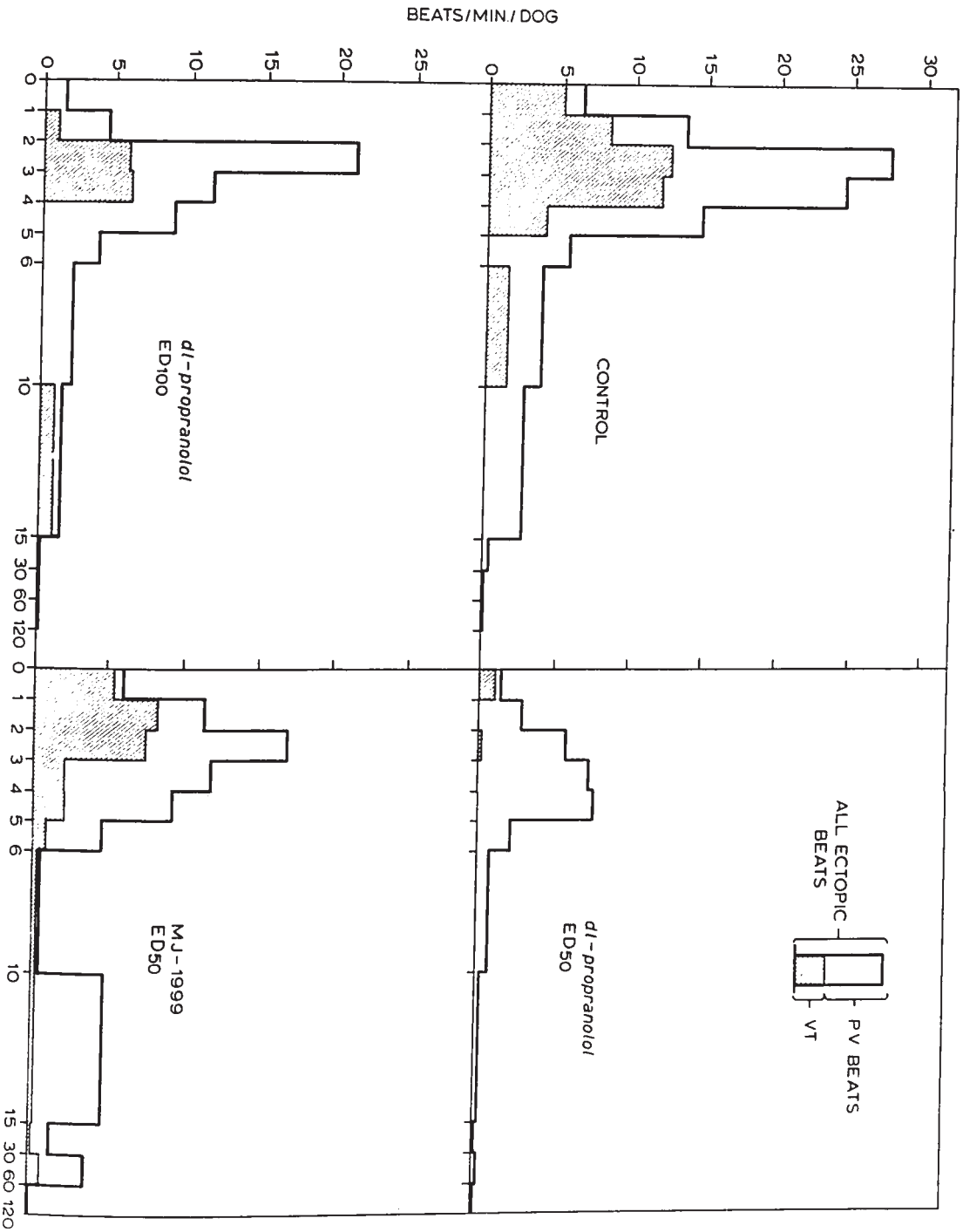

BEATS/MIN./DOG
CONTROL
dl-propranolol ED50
dl-propranolol ED100
MJ-1999 ED50
ALL ECTOPIC BEATS
PV BEATS
VT
TIME IN MINUTES FROM CORONARY OCCLUSION

Figure 16

Ventricular ectopic beats following sudden coronary occlusion in Groups 5, 6, 7 and 8.

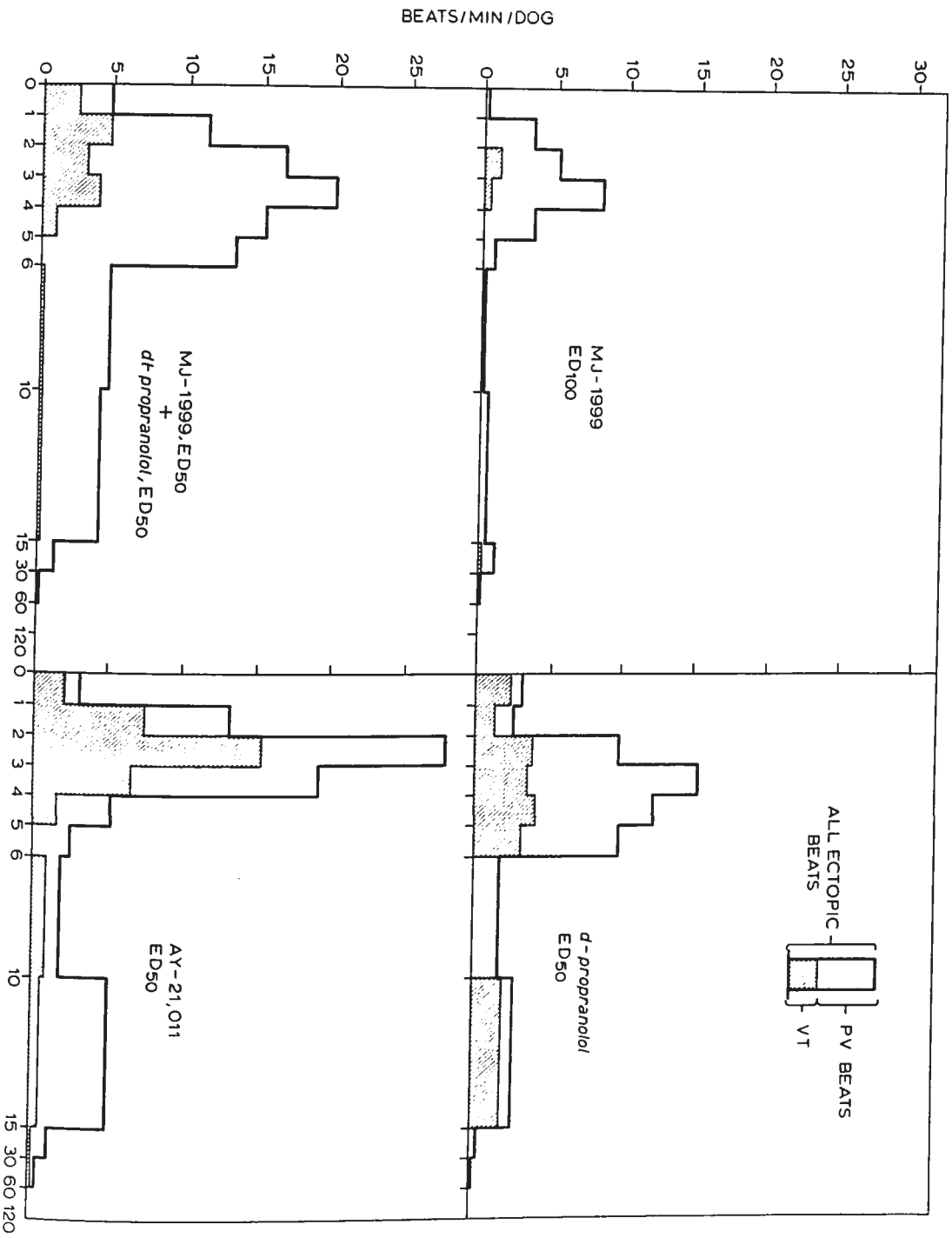

BEATS/MIN/DOG
MJ-1999
ED100
MJ-1999, ED50
+
dl-propranolol, ED50
d-propranolol
ED50
AY-21,011
ED50
ALL ECTOPIC BEATS
PV BEATS
VT
TIME IN MINUTES FROM CORONARY OCCLUSION

Table XI

Incidence of non-fatal ventricular ectopic beats following coronary occlusion. The difference between the number of dogs having premature ventricular beats following coronary occlusion in various groups is not significant ($p > 0.25$). The difference between the number of dogs showing ventricular tachycardia in various groups is significant ($p < 0.001$).

INCIDENCE OF NON-FATAL VENTRICULAR ECTOPIC BEATS

Group	Number of dogs	Premature ventricular beats		Ventricular tachycardia	
		Number	Percent	Number	Percent
1. Normal Saline	25	21	84	16	64
2. *dl*-propranolol, 0.1 mg/kg	25	17	68	3	12
3. *dl*-propranolol, 1.0 mg/kg	10	8	80	4	40
4. MJ-1999, 0.2 mg/kg	25	23	92	18	72
5. MJ-1999, 3.2 mg/kg	12	9	75	3	25
6. *d*-propranolol, 0.0723 mg/kg	16	11	68.8	6	37.5
7. MJ-1999, 0.2 mg/kg + *d*-propranolol, 0.0723 mg/kg	12	10	83.3	6	50
8. AY-21,011, 1.4 mg/kg	16	12	75	9	56.3

not statistically significant ($p > 0.25$). Ventricular tachycardia was noted in 12 to 72 percent of cases in various groups (Table XI). The difference in the incidence of this arrhythmia between various groups is significant ($p < 0.001$) and is due to the lower occurrence in *dl*-propranolol ED_{50} and MJ-1999 ED_{100} groups. The difference in the incidence of ventricular tachycardia between the control and the remaining groups is not significant ($p > 0.05$).

For numerical analysis, the ectopic beats were expressed in terms of their number per dog per minute during the three 5-minute periods immediately following coronary occlusion. The total number of ectopic beats per dog per minute, without regard to type ("all ectopic beats") during the first, second and third 5-minute periods is shown in Table XII. During the first five minutes, the difference in the frequency of "all ectopic beats" in the various groups is not significant by overall analysis of variance ($p > 0.05$). When the incidence of all ectopic beats in the control group is compared with all other groups taken together, the difference is significant ($p < 0.05$). This is due to the reduced incidence of "all ectopic beats" in Groups 2, 5 and 6. In the second and third 5-minute periods, the difference in the occurrence of all ectopic beats is not significant.

The number of "premature ventricular beats" per dog per minute during the first, second and third 5-minute

Table XII

The frequency of "all ventricular ectopic beats" per dog per minute during the first, second and third 5-minute periods following acute coronary occlusion. For each period, the frequencies of the various groups are not significantly different as determined by analysis of variance. The p values shown were determined by comparing each group with the control using student's t-test.

"ALL VENTRICULAR ECTOPIC BEATS" (±S.E.M.) PER DOG PER MINUTE

Group	First 5 Min		Second 5 Min		Third 5 Min	
	Number/Min	p	Number/Min	p	Number/Min	p
1. Normal Saline	16.06 ±3.3		4.18 ±2.13		2.26 ±2.09	
2. *dl*-propranolol, 01. mg/kg	5.12 ±1.38	<0.005	1.03 ±0.41	>0.1	0.2 ±0.1	>0.3
3. *dl*-propranolol, 1.0 mg/kg	11.32 ±5.00	>0.4	2.46 ±1.5	>0.3	1.49 ±1.01	>0.7
4. MJ-1999, 0.2 mg/kg	13.23 ±4.0	>0.5	2.31 ±1.27	>0.5	4.84 ±1.86	>0.3
5. MJ-1999, 3.2 mg/kg	4.72 ±1.59	<0.005	0.16 ±0.09	>0.05	0.42 ±0.32	>0.3
6. *d*-propranolol, 0.0723 mg/kg	6.51 ±2.92	<0.05	7.99 ±6.03	>0.5	22.53 ±21.89	>0.3
7. MJ-1999, 0.2 mg/kg + *d*-propranolol, 0.0723 mg/kg	13.02 ±4.26	>0.5	6.33 ±3.8	>0.5	4.24 ±2.37	>0.3
8. AY-21,011, 1.4 mg/kg	12.89 ±3.63	>0.5	1.4 ±0.82	>0.5	5.33 ±3.61	>0.4

periods is shown in Table XIII. The difference in their incidence is not statistically significant in any of the three 5-minute periods.

The difference in the occurrence of "ventricular tachycardia" during the first 5-minute period in the various groups is more clear cut and is statistically significant ($p < 0.05$). When the incidence of ventricular tachycardia in the control group is compared with that in all other groups taken together, the difference is also significant ($p < 0.01$). The incidence of this arrhythmia in the second and the third 5-minute periods is very low and was not subjected to any statistical test. This is illustrated in Table XIV.

2. First, second and third degree heart block

No dog in the Groups 1, 4, 6 and 7 had any atrio-ventricular block. Five out of 10 dogs treated with the higher dose of *dl*-propranolol developed progressive first, second and third degree atrio-ventricular block, three ending in cardiac standstill. Four out of 16 dogs treated with AY-21,011 developed progressive first, second and third degree heart block, two ending in cardiac standstill. Of the two dogs in the group treated with *dl*-propranolol (0.1 mg/kg) developing heart block, one ended in cardiac standstill. The incidence of severe heart block in Groups 3 and 8 is significantly higher than that in the control group ($p < 0.005$).

Table XIII

Frequency of premature ventricular beats during the first, second and third 5-minute periods following acute coronary occlusion. For each period, the frequencies of the various groups are not significantly different as determined by analysis of variance. The p values shown were determined by comparing each group with the control using student's t-test.

PREMATURE VENTRICULAR BEATS (± S.E.M.) PER DOG PER MINUTE

Group	First 5 Min		Second 5 Min		Third 5 Min	
	Number/Min	p	Number/Min	p	Number/Min	p
1. Normal Saline	5.44 ±1.44		3.09 ±1.43		2.26 ±2.09	
2. *dl*-propranolol, 0.1 mg/kg	4.26 ±1.18	>0.5	0.98 ±0.41	>0.1	0.20 ±0.10	>0.3
3. *dl*-propranolol, 1.0 mg/kg	7.87 ±2.65	>0.5	2.46 ±1.51	>0.7	0.51 ±0.22	>0.4
4. MJ-1999, 0.2 mg/kg	5.27 ±1.52	>0.9	2.06 ±1.10	>0.5	4.54 ±1.78	>0.4
5. MJ-1999, 3.2 mg/kg	4.47 ±1.54	>0.6	0.16 ±0.09	>0.05	0.42 ±0.32	>0.3
6. *d*-propranolol, 0.0723 mg/kg	3.53 ±1.56	>0.3	5.17 ±3.26	>0.5	3.20 ±2.57	>0.7
7. MJ-1999, 0.2 mg/kg + *d*-propranolol, 0.0723 mg/kg	10.65 ±3.55	>0.1	6.07 ±3.82	>0.4	4.04 ±2.25	>0.5
8. AY-21,011, 1.4 mg/kg	6.17 ±2.00	>0.7	0.68 ±0.34	>0.1	4.73 ±3.07	>0.5

Table XIV

The frequency of ectopic beats in the form of ventricular tachycardia per dog per minute during the first, second and third 5-minute periods following acute coronary occlusion. For the first 5-minute period, the difference between the frequencies of the various groups is significant as determined by analysis of variance ($p < 0.01$). The p values shown were determined by comparing each group with the control using student's t-test.

ECTOPIC BEATS IN THE FORM OF VENTRICULAR TACHYCARDIA PER DOG PER MINUTE

Group	First 5 Min		Second 5 Min	Third 5 Min
	Number/Min	p	Number/Min	Number/Min
1. Normal Saline	10.63 ±2.52		1.09	0
2. *dl*-propranolol, 0.1 mg/kg	0.86 ±0.76	<0.001	0	0
3. *dl*-propranolol, 1.0 mg/kg	3.45 ±2.83	>0.05	0	0.97
4. MJ-1999, 0.2 mg/kg	8.06 ±3.38	>0.5	2.36	0.30
5. MJ-1999, 3.2 mg/kg	0.25 ±0.19	<0.001	0	0
6. *d*-propranolol, 0.0723 mg/kg	2.98 ±1.74	<0.02	2.81	19.33
7. MJ-1999, 0.2 mg/kg + *d*-propranolol, 0.0723 mg/kg	2.36 ±0.97	<0.01	0.27	0.2
8. AY-21,011, 1.4 mg/kg	6.72 ±2.23	>0.25	0.62	0.6

3. Sinus arrest

No dog in the control group showed sinus arrest. Episodes of sinus arrests were noted in the remaining groups as follows: one dog in Group 4; two dogs in each of the Groups 2, 3, 5 and 6; three dogs in Group 8 and four dogs in Group 7.

4. Atrial tachycardia

This was noted in one dog in each of the Groups 1, 2 and 6.

5. Atrial fibrillation

Only one dog showed atrial fibrillation in this study. This was noted in a dog treated with *dl*-propranolol (0.1 mg/kg). Atrial fibrillation was noted 90 minutes after coronary occlusion.

6. Ventricular fibrillation

Ventricular fibrillation, in this study, was always fatal except in one dog in Group 4. This dog went into ventricular fibrillation 90 seconds after coronary occlusion but recovered spontaneously in six seconds and survived the total duration of observation of 24 hours.

7. Other findings

During the analysis of data, several features of interest were noted. These are: electrical alternans, mechanical alternans and post-extrasystolic T-wave changes. These are illustrated in Figure 17.

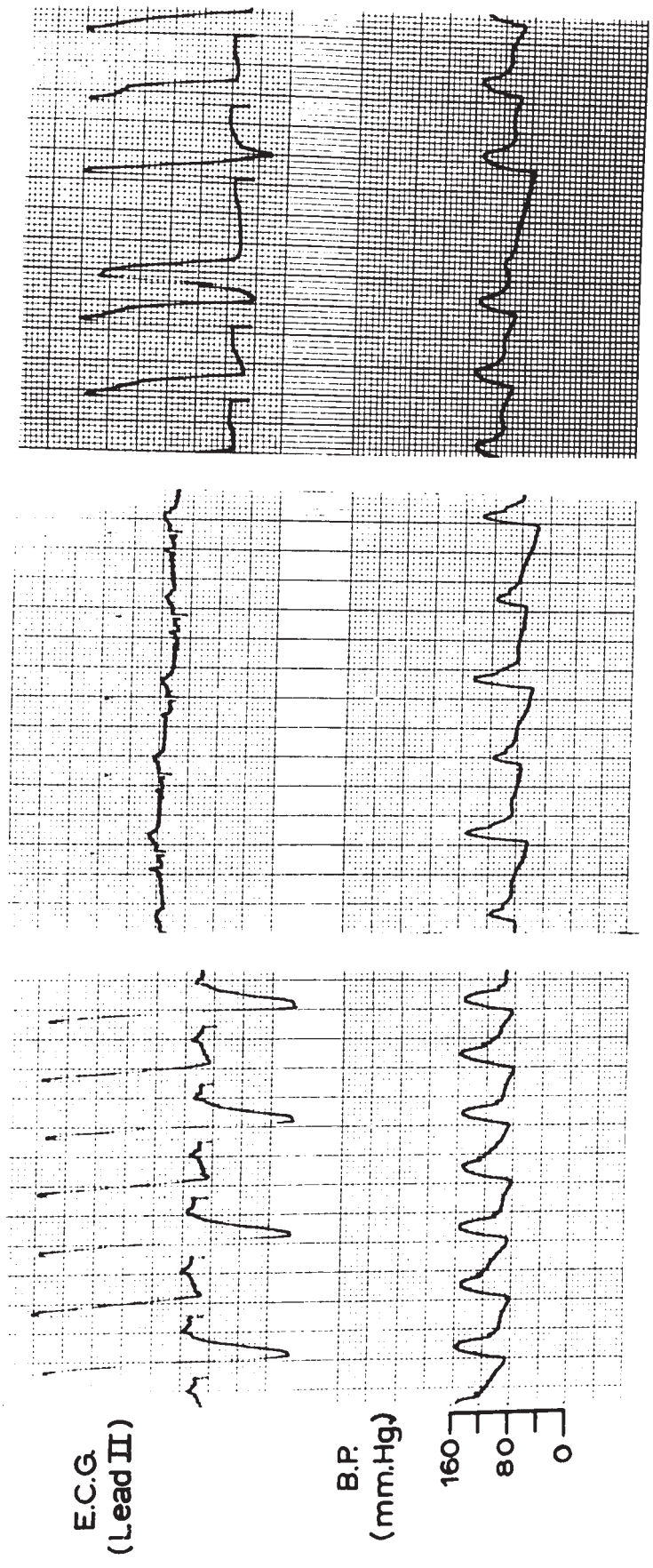
Figure 17

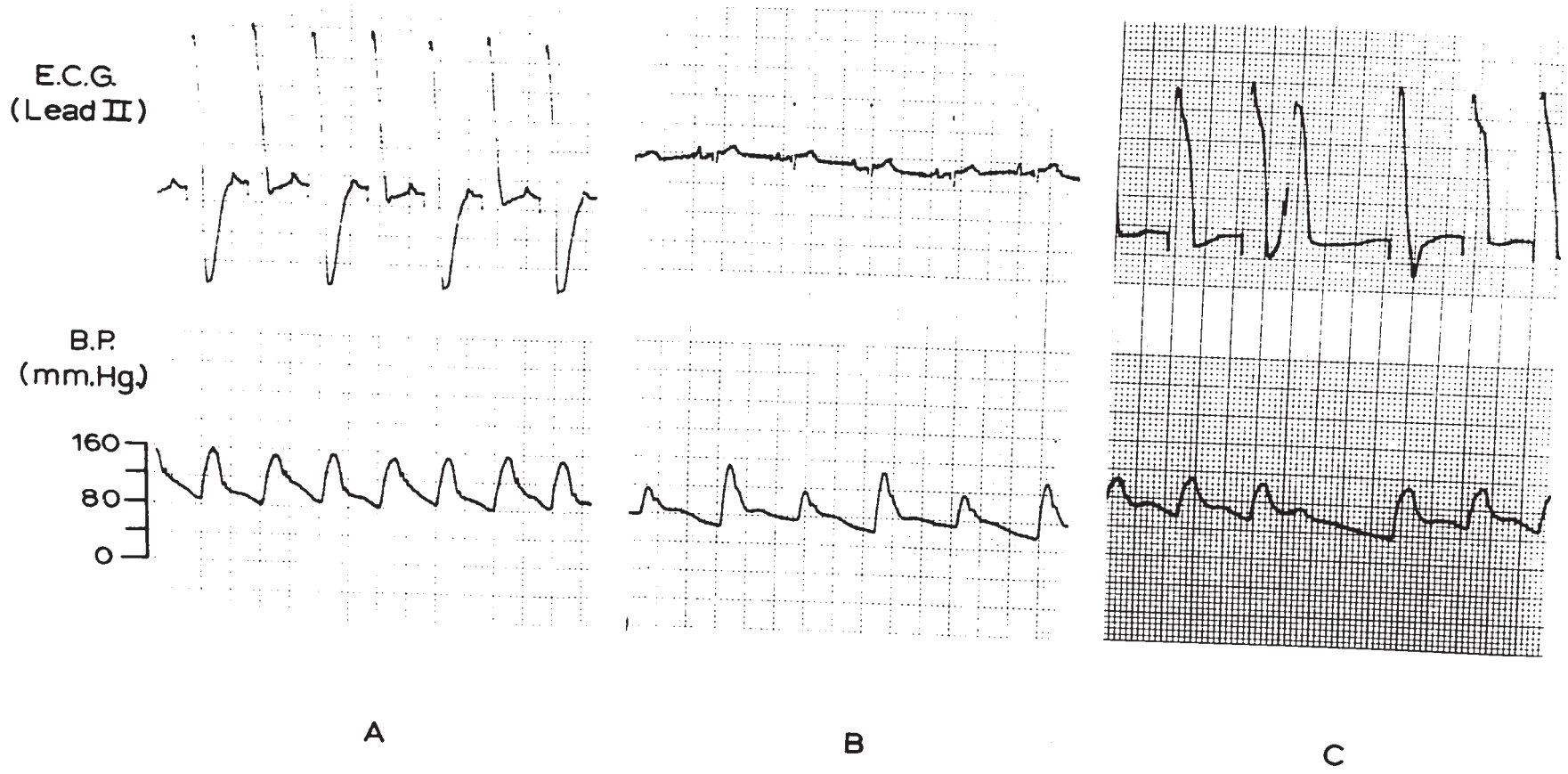
Electrical alternans, mechanical alternans and post-extrasystolic T-wave change.

A. Electrical alternans in a Group 2 dog one minute after coronary occlusion.

B. Mechanical alternans in a Group 3 dog 90 seconds after coronary occlusion.

C. Post-extrasystolic T-wave accentuation in a Group 5 dog 3.5 minutes after coronary occlusion.


E.C.G.
(Lead II)
B.P.
(mm.Hg)
160
80
0
A
B
C


E.C.G.
(Lead II)
B.P.
(mm.Hg.)
160
80
0
A
B
C

VII. Pathological Examination

Occlusion of the circumflex branch of the left coronary artery led to a large infarct involving approximately 50 percent of the left ventricular wall. The affected area was the posterolateral surface of the left ventricle encroaching on the posterior part of the interventricular septum and the adjoining area of the right ventricular wall. Anteriorly, it regularly extended up to the anterior papillary muscle. The infarcted area was clearly delineated from the non-infarcted area and extended from the apex to the atrioventricular ring. A thin rim of normal tissue, 1-2 mm in thickness, in the subepicardial area usually covered the infarcted area. Figure 18 shows the gross findings in a typical case.

VIII. The Rejected Cases

Thirteen experiments were unacceptable due to reasons cited in Table XV. In five experiments, the ligature around the circumflex branch of the left coronary artery cut through the wall of the artery when the cord was pulled. These animals died within 10 minutes of pulling the cord with progressive hypotension due to bleeding from the proximal end of the torn artery. Three experiments were rejected because of a lack of electrocardiographic change compatible with transmural myocardial infarction. In each of these cases there were only transient T wave changes with the pulling of the ligature. Examined 24 hours later, these animals showed ischemic T wave changes but

Figure 18

Gross findings in the heart 24 hours after coronary occlusion in a Group 7 dog.

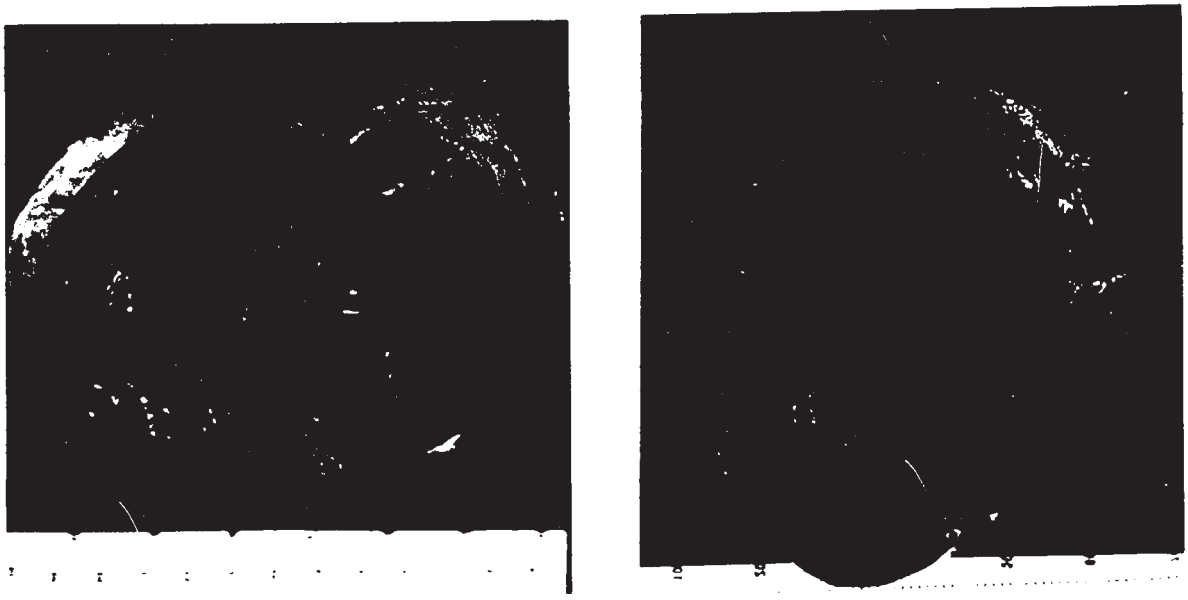
A. Front view of the heart.

B. Sections of the same heart showing the extent of the pale infarct.

N.B. Similar gross findings were observed in all groups of animals when they survived this length of time.

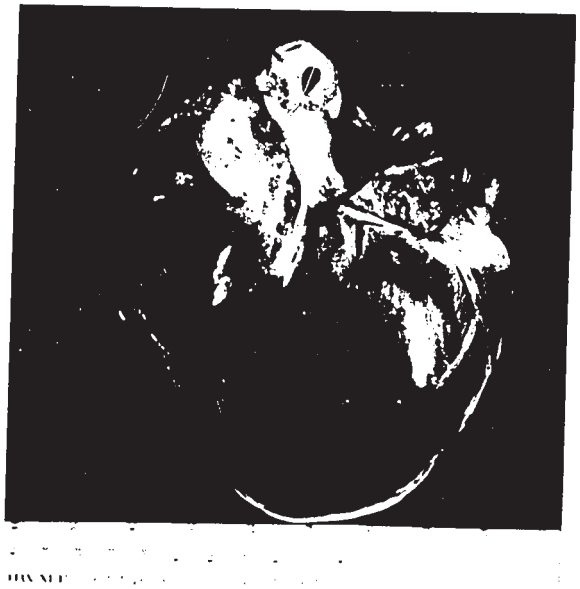
(A)

Posterior



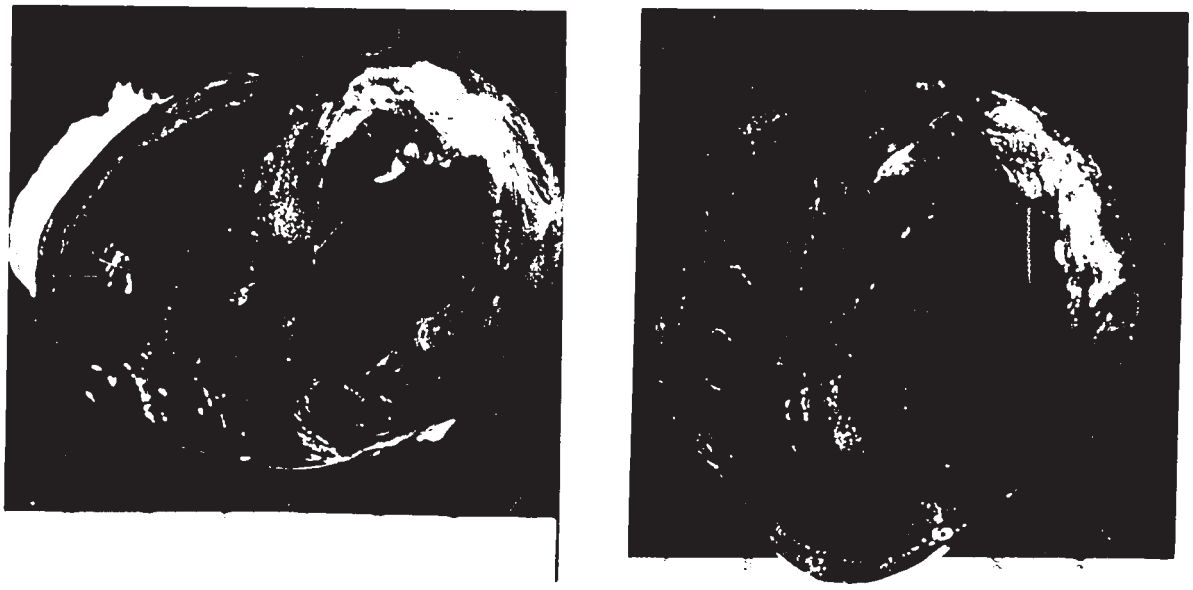
Anterior

(B)



(A)

Posterior



Anterior

(B)

Table XV

Causes of rejection of animals from the study.

THE REJECTED CASES

Serial No.	Experimental Group	Outcome	Reason for Rejection
1.	*dl*-propranolol, ED_{50}	Dead	Torn coronary artery.
2.	MJ-1999, ED_{50}	Dead	Death due to hemorrhage
3.	MJ-1999, ED_{50}	Dead	from the proximal end of
4.	AY-21,011, ED_{50}	Dead	the torn artery.
5.	*d*-propranolol, ED_{100}	Dead	
6.	Control	Alive	Lack of ECG changes on the
7.	Control	Alive	day of experiment and also
8.	MJ-1999, ED_{50} + *dl*-propranolol 0.0723 mg/kg	Alive	24 hours later. Only sub-endocardial infarction at autopsy.
9.	Control	Dead	Incomplete occlusion of the
10.	MJ-1999, ED_{100}	Alive	circumflex artery.
11.	MJ-1999, ED_{100}	Alive	Missed several branches of
12.	Control	Dead	the circumflex artery.
13.	MJ-1999, ED_{50}	Dead	Hypertension due to congenital polycystic kidneys.

no QRS changes diagnostic of myocardial infarction. Autopsy revealed free-flowing anastomoses between the smaller branches of the anterior descending branch and the distal branches of the circumflex branch, the latter filling from the former. Gross examination of the heart showed only subendocardial myocardial infarction. Two experiments were rejected because of incomplete occlusion of the circumflex branch with leakage through the ligated area. These two cases showed subendocardial infarction at autopsy. The ligatures were placed too far away from the bifurcation of the left coronary artery missing several branches of the circumflex branch in two cases which were also rejected. One dog was found to have hypertension due to congenital polycystic kidneys. This case was also excluded from the study.

Regrouping the rejected cases into those which went in favor of the conclusion drawn from the acceptable cases, and those which went against it, shows that seven experiments went against and six in favor of the final conclusion. So the rejected cases seem to have no bearing on the final conclusions drawn from 141 accepted experiments.

DISCUSSION

Comparison of data from experimental coronary occlusion studies by different investigators is not always easy. The mortality rate resulting from ligature of coronary arteries, reported in the literature, varies from 0 to 100 percent (Allen and Laadt, 1950). The reasons for this wide range are many, but several of these variables are controllable.

Anesthesia, thoracotomy, and artificial respiration have two major implications on the results of experimental coronary occlusion. Firstly, they all modify cardiac functions and as such, the results of experiments on anesthetized, open-chest animals are not necessarily applicable to cardiovascular responses in intact, unanesthetized, "normal" animals. Secondly, these factors not only have profound circulatory effects of their own, but will influence the circulatory responses to the pharmacological agents being studied.

It has been recognized for a long time that the mortality rate from experimental coronary occlusion in dog is lower if the animal is anesthetized (Manning, McEachern and Hall, 1939; Manning, 1948; Olichney and Modell, 1958; Hoffmeister, Regelson and Rubin, 1958). Different anesthetic agents may modify the results, because their action on circulation may be different. Pentobarbital sodium increases the heart rate and decreases the cardiac output and blood pressure (Olmsted and Page, 1966). Alpha chloralose anesthesia in dogs increases the heart rate and blood pressure (Bass and Buckley, 1966) and disturbs the autonomic nervous system reflexes in an

unpredictable manner (Croft, 1964). Dogs anesthetized with urethane have a higher resting cardiac output, pulmonary venous and arterial pressures and a lower heart rate than dogs anesthetized with pentobarbital (Giles and Burch, 1970). Variations in the depth of anesthesia have been responsible for the inconsistent circulatory effects in many experiments.

Many coronary occlusion studies have been performed on open-chest animals (Pentecost and Austen, 1966; Ceremuzynski *et al.*, 1969). Thoracotomy in dogs results in tachycardia and a reduction in the heart size, cardiac output and blood pressure (Ferguson, Shadle and Gregg, 1953; Rushmer, Finlayson and Nash, 1954). Positive-pressure respiration in anesthetized dogs reduces cardiac output and stroke volume (Morgan, Martin, Hornbein, Crawford and Guntheroth, 1966; Holt, 1954). Pentecost and Austen (1966), after demonstrating that propranolol (0.08 mg/kg) reduced the incidence of ventricular fibrillation following coronary occlusion in open-chest dogs with positive-pressure respiration, considered the possibility that the beneficial effect might have been related to the prevention of the 'oxygen-wasting' tachycardia after thoracotomy.

The mode of coronary occlusion has also an important bearing on the outcome and creates some problems in comparing the results from different investigators. Harris (1950) observed that the one-stage ligation of the anterior descending artery in dogs led to ventricular fibrillation in three out of four dogs. This led to his development of the two-stage technique by which the artery was constricted but not

totally occluded in the first stage and, 30 minutes later, in the second stage, the artery was totally occluded. Adoption of the two-stage technique for coronary occlusion resulted in only one ventricular fibrillation in 60 dogs (Harris, 1950). The technique (Manning, McEachern and Hall, 1939) for coronary occlusion, used in the present study includes the early phase of ventricular irritability following coronary ligation and, therefore, is associated with higher early mortality rate. Gradual coronary artery constriction by ameroid constrictors, on the other hand, leads to sudden death due to ventricular fibrillation over a protracted period of two to three days (Vineberg, Mahanti and Litvak, 1960; Peter, Rau, Whalen, Entman and McIntosh, 1966; Hurst, Morris, Zeft, Hackel and McIntosh, 1967; Zeft, Whalen, Ratliff, Davenport and McIntosh, 1968).

The mortality rate also varies with the artery ligated and the level of occlusion along each artery. The death rate is higher when the circumflex branch is occluded than that following the occlusion of the anterior descending branch (Manning, 1948). Allen and Laadt (1950) pointed out that an important variable in the outcome of the ligation of the circumflex branch was the level at which it was occluded. Thus, the 24-hour mortality rate following circumflex branch ligation varied from 36 to 82 percent depending on how many branches of this artery escaped ligation or from 30 to 84 percent depending on the distance of the ligature from the origin of the circumflex artery; the closer the ligature to the bifurcation of the left coronary artery, the higher the mortality rate.

Although the disadvantages of using anesthetized dogs for coronary occlusion studies were pointed out as early as 1939 (Manning *et al.)*, it is only in recent years that the conscious dog has been the subject of hemodynamic investigations. To avoid the influences of anesthesia, thoracotomy and positive-pressure respiration, fully conscious dogs were used in the present study. While others (Manning *et al.*, 1939; McEachern *et al.*, 1940; Manning, 1948; Allen and Laadt, 1950; Laadt and Allen, 1950; Hoffmeister *et al.*, 1958; Regelson *et al.*, 1959; Skelton *et al.*, 1962; Skelton, 1961) allowed only one day between the operative placement of the ligature around the coronary artery and the pulling of the ligature to occlude it, 48 to 72 hours were allowed in the present investigation to ensure better recovery from the first stage operation, under pentobarbital anesthesia. Comparison of the pH and blood gas analysis between the experimental animals and a group of nine non-thoracotomized normal dogs strongly suggests that this period of waiting after the operative placement of the ligature was quite adequate.

The above considerations led us to believe that the left circumflex artery was the vessel of choice in coronary occlusion studies. Ligation of this artery, by cutting off nearly 50 percent of the total blood supply of the heart, provided a major challenge to the survival of the dogs (Manning *et al.*, 1939) and allowed for tne assessment of the protective measures against the fatal outcome. Circumflex branch ligation has been claimed to provide more uniform results compared to the ligation of the anterior descending branch (Skelton, 1961; Fineberg, Scicchitano and Camishion, 1962; Fineberg,

Foris and Camishion, 1962). The uncertain outcome from ligation of the anterior descending branch in the dog is mainly due to a great variation in the origin of the septal artery. While in the human the blood supply to the interventricular septum is provided by four to six equally large branches of the left anterior descending artery, in the dog there is a large first septal branch with smaller penetrating arteries from anterior and posterior descending arteries (James, 1961). Up to 75 percent of all blood to the interventricular septum has been said to come through the first septal artery, the origin of which cannot always be identified in the beating heart (Fineberg *et al.*, 1962). The mortality rate resulting from occlusion of the anterior descending branch would vary depending on whether or not the first septal branch has been occluded. The two-hour and 24-hour mortality rates of 72 percent and 84 percent in the control group of the present investigation are within the range reported by others using similar methods. Thus the one-hour mortality rates have been reported to vary from 57 to 80 percent and 24-hour mortality rate, from 81 to 84 percent (Laadt and Allen, 1950; Allen and Laadt, 1950; Skelton *et al.*, 1962). In other reports where the location of ligature on the circumflex artery was either not mentioned or placed several millimeters away from the bifurcation, the one-hour mortality varied from 48 to 67 percent and the 24-hour mortality from 52 to 84 percent (Skelton, 1961; Manning, 1948; Regelson *et al.*, 1959, LeRoy *et al.*, 1942).

In experiments using mongrel dogs, it is almost impossible to know the exact age of the dogs used. Manning (1948) has demonstrated

that older dogs do not tolerate coronary occlusion as well as younger animals. In the present investigation, only healthy adult dogs were used; very old and very young dogs were avoided.

Once it was decided to study the influence of beta-adrenergic receptor blocking drugs in experimental coronary occlusion, the first task was to determine the appropriate doses of these agents. The doses of these drugs used in the few reported studies had no obvious justification. There is, in fact, no simple and reliable way to determine the extent of beta-blockade achieved in an intact animal by a particular dose of one of these agents. The level of beta-blockade depends on the degree of sympathetic and vagal tones present at the time of testing and also on the dose and potency of the drug used and on additional pharmacological properties that these drugs may possess. Another problem, in this regard, is the competitive nature of blockade by these agents (Fitzgerald, 1969). The method of testing the potency of beta-blocking drugs by their ability to reduce the tachycardia following a challenge by a standard dose of exogenous isoproterenol has led to some confusion. Thus Proger *et al.* (1966), in their coronary occlusion studies, used a slow infusion of *dl*-propranolol 'calculated to maintain 100 percent blockade'. Using isoproterenolol in a challenge dose of 0.02 mg (less than 0.3 µg/kg for a 70 kg person), Frankl and Soloff (1968) concluded from their studies in human volunteers that 'Sotalol in doses sufficient to block isoproterenol-induced beta-adrenergic receptor stimulation did not reduce cardiac output or cardiac work below control values'. These statements are only justified for the dose levels considered as the

levels of blockade could well have been overcome by higher doses of isoproterenol.

To determine the equiactive doses of the drugs used in the present study, we used the antagonism by these drugs of the tachycardia evoked by 1.5 μg/kg of isoproterenol. This challenging dose of isoproterenol was reasonably high and its effect disappeared in 15 to 20 minutes. The calculated doses from the regression lines of *dl*-propranolol and MJ-1999 used in the present study are equiactive as far as the antagonism of the dose of isoproterenol is concerned. It is not being claimed that the calculated ED_{50} and ED_{100} doses of these two drugs caused or maintained 50 and 100 percent blockade of the beta receptors in conscious dogs.

Propranolol has been claimed to be eight to 16 times as potent as MJ-1999 (Fitzgerald, 1969; Gomoll, 1970). It must be noted that these studies were performed on anesthetized animals or isolated preparations. Our data show that *dl*-propranolol is only twice as potent as MJ-1999 at ED_{50} dose and three times as potent as MJ-1999 at ED_{100} dose. Since we were to use conscious animals in our studies, we elected to use the doses that we found equiactive in conscious animals. Very recently Ekue, Lowe and Shanks (1970) reported *dl*-propranolol to be 2.67 times as potent as MJ-1999 in antagonizing the chronotropic effect of isoproterenol in conscious men. It is indeed reassuring to find such excellent quantitative similarity between conscious man and dog. Our study, together with this report, show the need for extreme care in overgeneralization in comparing the results obtained at different centres based on different methodology.

Accurate calculation of potency ratio for AY-21,011 with respect to other beta-blocking drugs in conscious dogs is difficult (Dunlop and Shanks, 1968). Progressive reduction by AY-21,011 of isoproterenol-induced tachycardia which was found in anesthetized dogs was not found in conscious dogs. The dose response curve for AY-21,011 was also much flatter than those of *dl*-propranolol and MJ-1999 (Dunlop and Shanks, 1968) in anesthetized dogs. In anesthetized dogs 6.4 mg/kg of this drug produced 97 percent blockade of isoproterenol-induced tachycardia but in conscious dogs this dose produced only 60 percent blockade. After the administration of pempidine or atropine, AY-21,011 caused progressive blockade of the chronotropic effect of isoproterenol in anesthetized dogs (Dunlop and Shanks, 1968; Barrett *et al.*, 1968). It has been suggested that the increase in heart rate by isoproterenol in conscious dogs results from two mechanisms - a direct effect of the drug on the sino-atrial node and a reflex reduction in vagal inhibition in response to the fall in arterial blood pressure by isoproterenol. Because AY-21,011 did not block the latter effect, it only produced a partial block of isoproterenol-induced tachycardia. Propranolol and MJ-1999 abolished both direct and reflex effects and thus completely prevented the increase in heart rate. After treatment of conscious dogs with atropine or pempidine to abolish the reflex component of the isoproterenol-tachycardia, AY-21,011 produced almost complete blockade of the effect of isoproterenol on the heart rate.

With the above problems in mind we elected to determine the dose-response curve for AY-21,011 in conscious dogs treated with

atropine sulphate, 0.2 mg/kg. While Dunlop and Shanks (1968) found AY-21,011 to be one-third to one-fourth as potent as *dl*-propranolol, our studies in conscious dogs treated with atropine proved it to be only one-fourteenth as potent as *dl*-propranolol. The calculated ED_{50} dose of AY-21,011 of 1.4 mg/kg from our experiments done on atropinized conscious dogs is most likely not equivalent to the ED_{50} doses of *dl*-propranolol and MJ-1999, because these doses were determined from experiments in non-atropinized conscious dogs. Since with the administration of atropine sulphate the sympathetic nervous system was unopposed, the calculated ED_{50} dose of 1.4 mg/kg for AY-21,011, in all likelihood, caused a higher degree of blockade than produced by the calculated ED_{50} doses of 0.1 mg and 0.2 mg/kg for *dl*-propranolol and MJ-1999 respectively. This is supported by the fact that two out of five deaths in the group treated with 1.4 mg/kg of AY-21,011 were due to cardiac standstill. Though most likely empirical, this dose was used because others have used similar doses for the treatment of experimental cardiac arrhythmias. Thus Dunlop and Shanks (1968) observed that 1 mg/kg was as effective as 3 mg/kg of AY-21,011 in reducing or preventing the ectopic response to adrenaline in conscious dogs on second, third and fourth days after coronary ligation.

The flatness of the dose-response curve for antagonism of isoproterenol-induced tachycardia by AY-21,011 is well known (Dunlop and Shanks, 1968). In the present study, the dose-effect curve for MJ-1999 was flatter than that for *dl*-propranolol ($p < 0.05$). This is possibly associated with a concomitant direct cardiac depression by *dl*-propranolol more so than by MJ-1999 (Blinks, 1967: Hoffman and

Grupp, 1969).

The effects of the drugs, used in the present study, on the cardiovascular system are those that were expected from the results of others (Weiser *et al.*, 1966; Lish *et al.*, 1967; Kaumann and Aramendia, 1968; Barrett, 1969; Jewitt *et al.*, 1970). The extent of the reduction in the heart rate in these experiments is, however, not as marked as reported previously. Although these drugs as a group reduced the heart rate, this reduction was statistically significant with only the lower dose of MJ-1999 and the two dose-levels of *dl*-propranolol. Despite the fact that AY-21,011 possesses a mild intrinsic sympathomimetic effect and has been reported to increase the heart rate in anesthetized cats (Dunlop and Shanks, 1968), in most clinical studies there was reduction in the heart rate following its administration (Gibson and Sowton, 1968; Gibson, Balcon and Sowton, 1968; Sowton *et al.*, 1968; Fitzgerald and Scales, 1968).

The rise of blood pressure following the administration of these drugs is explained by an exaggerated alpha-receptor response of the peripheral blood vessels to catecholamines (Garret, Malafaya-Baptista and Osswald, 1966) and the resultant increase in the peripheral resistance (Weiser *et al.*, 1966). The fact that the larger doses of *dl*-propranolol and MJ-1999 caused lesser elevation than the lower doses is most likely due to a greater fall in cardiac output resulting from the higher doses than the lower ones. The only discernable change in the electrocardiogram by the drugs used in the present study was noted in the PR interval which was significantly prolonged by both dose levels of *dl*-propranolol and MJ-1999 and by *d*-propranolol but not by

AY-21,011. Intrinsic sympathomimetic action of the latter drug seems to be the explanation for this effect on the PR interval. These results are similar to those reported by others (Barrett, 1969; Hoffman and Grupp, 1969).

The rise in the heart rate and the fall in the blood pressure noted following acute coronary ligation were not significantly negated by the beta-blocking drugs in the present study.

Pre-treatment with *dl*-propranolol (0.1 mg/kg) in our study was shown to decrease significantly the two-hour and the 24-hour mortality rate when compared with the control group. However, this drug, given in a dose of 1.0 mg/kg did not exhibit such protection. While not exactly comparable, these results are similar to those reported by Pentecost and Austen (1966) who observed 50 percent death rate with a higher dose (0.2 mg/kg) and only 14 percent death rate with a lower dose (0.08 mg/kg) in anesthetized open-chest dogs. The higher mortality rate in dogs receiving the higher dose of *dl*-propranolol seemed to these investigators to be due to greater depression of myocardial contractility as evidenced by a profound reduction in cardiac output and mean arterial pressure and a greater reduction in the mean systolic ejection rate than in the dogs receiving the lower dose. According to Barrett (1969), *dl*-propranolol reduces cardiac work by blocking sympathetic drive to the heart at doses up to 0.2 mg/kg and direct depression of myocardium only occurs at doses well above this. However, slightly higher doses have been used by some investigators with good results. Proger *et al.* (1966), using a higher dose (0.31 mg/kg followed by slow infusion) of *dl*-propranolol

observed a significant protective effect of this drug against ventricular fibrillation in anesthetized dogs. Suppression of arrhythmia after coronary ligation has also been noted following the administration of *dl*-propranolol, 0.2 to 0.3 mg/kg, in open-chest dogs (Ceremuzynski *et al.*, 1969). Although there is no reported study of *dl*-propranolol in conscious dogs, it is highly probable that the higher dose of *dl*-propranolol in our study was cardiodepressant. More dogs in the group pre-treated with the higher dose of *dl*-propranolol demonstrated evidence of depression of the conduction tissue following coronary occlusion: more dogs showing sinus arrests and heart blocks. Three of seven two-hour deaths in this group were due to cardiac standstill compared with only one out of six two-hour deaths in the group treated with the lower dose. The group receiving the higher dose of *dl*-propranolol also demonstrated a higher number of ectopic beats, compared with the group receiving the lower dose. The mechanism of this arrhythmia may be similar to that following the administration of quinidine (Moe and Abildskov, 1970; Friedberg, 1966).

While the lower dose of *dl*-propranolol was more beneficial than the higher dose in experimental coronary occlusion, this did not hold true for the two dose levels of MJ-1999. The mortality was significantly reduced with both doses (0.2 mg/kg and 3.2 mg/kg) of the latter drug. There is no reported study on the influence of MJ-1999 in experimental coronary occlusion in conscious dogs to compare with our study. The report of Kaumann and Aramendia (1968) involving the ligation of the anterior descending branch in anesthetized dogs is not quite comparable with our work. These investigators reported

protective effect against ventricular fibrillation following experimental coronary occlusion by MJ-1999 in doses of 10 mg/kg, but not in doses of 0.5 mg/kg. The higher mortality rate in the group treated with 1.0 mg/kg of *dl*-propranolol compared with that in the group treated with equiactive dose of 3.2 mg/kg of MJ-1999, in our study, is most likely due to greater direct myocardial depressant action of the former drug (Blinks, 1967; Hoffman and Grupp, 1969). Whether the greater degree of direct myocardial depression by *dl*-propranolol than by MJ-1999 is related to the 'quinidine-like effect' possessed by the former and not by the latter, is controversial. Thus Levy (1967, 1968), using isolated electrically driven or spontaneously beating rabbit atria, found that the negative inotropic and chronotropic effects of 20 beta-blocking drugs were independent of their beta-adrenergic blocking potency; that the optical isomers of any given drug, while differing in beta-blocking potency, still showed essentially the same negative inotropic and chronotropic effects; and that the negative inotropic effect of appropriate concentrations of beta-blocking drugs was evident even in hearts depleted of norepinephrine and therefore concluded that the negative inotropic effect of this class of compounds was related to the "quinidine-like" action acting in a non-beta-blocking fashion. On the other hand, Barrett (1969) has demonstrated that in thiopentone/chloralose anesthetized dogs, while 0.25 mg/kg of *dl*-propranolol caused 23 percent drop in heart rate, 18 percent drop in cardiac contractile force, 15 percent drop in tension time index and seven percent drop in ejection rate, *d*-propranolol which is devoid of beta-blocking effect and has

equal membrane-effect, in the same dose, caused two percent reduction in heart rate, six percent drop in cardiac contractile force, six percent increase in tension time index and two percent increase in ejection rate. In doses of 1.25 and 5.25 mg/kg, although *dl*-propranolol caused greater reduction of cardiac contractile force than *d*-propranolol, the difference was not statistically significant. At the higher doses the racemic compound produced statistically significant greater reduction in the heart rate and tension time index. The author concluded that the negative inotropic effect of *dl*-propranolol was not due to its 'quinidine-like' effect but was due to the effect of beta-adrenergic receptor blockade *per se*.

The fact that the ED_{50} doses of MJ-1999 and *dl*-propranolol, in the present study, afforded similar protection against ventricular fibrillation following acute coronary occlusion in conscious dogs, that *dl*-propranolol possesses both beta-adrenergic blocking effect and local anesthetic effect (Barrett and Cullum, 1968; Levy, 1968) and that MJ-1999 has only beta-adrenergic receptor blocking property without any local anesthetic action (Somani and Lum, 1965; Somani *et al.*, 1966; Lish and Soloff, 1968; Levy, 1968), strongly suggest that the beneficial effect of this group of drugs, in the doses used in our experiments, is primarily due to beta-adrenergic receptor blockade and not the result of the local anesthetic effect. This conclusion is further supported by the fact that *d*-propranolol, which is practically devoid of beta-blocking effect, in a dose having equiactive membrane-effect as that of the ED_{50} dose of *dl*-propranolol, afforded no protection against ventricular fibrillation (*d*-propranolol

69 percent mortality compared to *dl*-propranolol 24 percent mortality and control 72 percent mortality). This does not imply that *d*-propranolol, in a suitable dose, will not prevent ventricular fibrillation following experimental coronary ligation. On the contrary, it is highly likely that it will. Local anesthetic agents are well known for their antiarrhythmic effect and Stephenson, Cole, Parrish, Bauer, Johnson, Kochtitzky, Anderson, Hibbit, McCarty, Young, Wilson, Meiers, Meador, Ball and Meneely (1960) have demonstrated that procaine at a dose of 4.6 mg/kg is capable of significantly preventing ventricular fibrillation following temporary occlusion of the anterior descending branch for 30 minutes.

The present investigation further suggests that it is not necessary to block the beta-adrenergic receptors throughout the entire body to protect the heart from fibrillation following coronary ligation. Selective blockade of the beta receptors in the heart alone is sufficient to achieve this benefit. Cardio-selective beta-adrenergic receptor blockade was made possible by the development of AY-21,011 (ICI 50,172; Practolol). As stated above, this drug is devoid of local anesthetic effect and has mild intrinsic sympathomimetic activity. Pre-treatment with AY-21,011 significantly reduced the incidence of ventricular fibrillation and of death following coronary occlusion in conscious dogs. There is no published report available where AY-21,011 was used in experimental coronary occlusion in conscious dogs to compare with our study. However, the antiarrhythmic effect of this drug has been demonstrated in three anesthetized open-chest dogs following coronary ligation by Ceremuzynski *et al.* (1969). Jewitt *et al.* (1970) observed that the frequency of ventricular ectopic

beats was reduced in seven out of 15 patients with acute myocardial infarction.

The decreased mortality rate from acute coronary occlusion in conscious dogs by appropriate doses of the beta-blocking drugs in the present study parallels that found in other studies involving chemical or surgical sympathectomy (McEachern *et al.*, 1940; Manning, 1948; Milch *et al.*, 1955; Hoffmeister *et al.*, 1958; Skelton, 1961; Skelton *et al.*, 1962). The procedure which has proved to be successful in the present investigation has the added advantage of being cardio-selective, if AY-21,011 is used.

The temporal distribution of ventricular ectopic beats following sudden coronary ligation in the present study is in agreement with a very important observation made by Harris, Estandia and Tillotson (1951). These authors described three phases of ectopic beats after coronary occlusion in anesthetized dogs. The first phase lasted for about 10 minutes and was the period of maximum frequency of ectopic beats frequently leading to ventricular fibrillation. In the second phase lasting about eight hours, there were very few ectopic beats which occurred again in the third phase progressing to ventricular tachycardia but not ventricular fibrillation. Although we did not electrocardiographically monitor our surviving animals beyond two hours from coronary occlusion, our findings in conscious dogs do not disagree with these observations. When present, ventricular ectopic beats usually appeared within two minutes and reached peak frequency between two and five minutes of coronary occlusion. The frequency of ectopic beats then gradually declined with rare ectopic beats beyond

15 minutes. Almost all deaths due to ventricular fibrillation occurred during the first 15 minutes. This period corresponded with the first phase described by Harris *et al.* (1951). We monitored our animals for one to two hours following coronary occlusion. The markedly reduced or the absence of ectopic beats from 15 minutes to the end of our monitoring period corresponded with part of the second phase described by Harris *et al.* When an electrocardiogram was done on surviving animals, 24 hours after coronary occlusion, an overwhelming majority demonstrated multifocal ectopic beats. This corresponded with the third phase of Harris' description. From our study, we are unable to tell if the duration of these three phases in conscious dogs is similar to those described by Harris *et al.* (1951). Multiple factors are probably involved in the production of ectopic impulses in each of these three phases. Direct and indirect evidence suggest that the catecholamines and the sympathetic nerves to the heart are important in the genesis of ectopic beats and of ventricular fibrillation during the early phases (McEachern, Manning and Hall, 1940; Skelton, 1961; Regelson *et al.*, 1959; Milch *et al.*, 1955; Manning and Caudwell, 1947; Clark and Cummings, 1957; Jewitt *et al.*, 1969; Staszewska-Barczak and Ceremuzynski, 1968; Ceremuzynski *et al.*, 1969). Delayed ventricular ectopic beats after coronary occlusion, though increased in frequency by catecholamines (Clark and Cummings, 1957; Maling and Moran, 1957), are possibly predominantly linked with potassium liberated from necrotizing heart tissue (Harris *et al.*, 1954) and are not primarily related to sympathetic activity. The latter statement is supported by the lack of

influence of dichloroisoproterenol (Moran *et al.*, 1962), pronethalol (Somani and Lum, 1965) and *dl*-propranolol (Shanks and Dunlop, 1967) on the ectopic ventricular beats occurring 24 hours after experimental coronary occlusion. On the other hand, beta-blocking drugs such as *dl*-propranolol (Pentecost and Austen, 1966; Proger *et al.*, 1966; Ceremuzynski *et al.*, 1969), MJ-1999 (Kaumann and Aramendia, 1968) and AY-21,011 (Ceremuzynski *et al.*, 1969) have been reported to suppress cardiac arrhythmia of the early phase in anesthetized dogs. Recognition of the phases described by Harris *et al.* (1951) thus seems to be important in the interpretation of the diverse results reported by different investigators.

In our investigation using conscious dogs, it was found that the beta-blocking drugs prevented serious arrhythmias during the early phase following coronary occlusion. Ventricular fibrillation was significantly reduced by the lower dose of *dl*-propranolol, by both dose levels of MJ-1999, by the lower dose of MJ-1999 in combination with *d*-propranolol (in equiactive local anesthetic ED_{50} dose of *dl*-propranolol) and by AY-21,011. With the higher dose of *dl*-propranolol, 40 percent incidence of ventricular fibrillation contrasted with 72 percent incidence in the control group approaches the level of significance ($p < 0.1 > 0.05$) in spite of the small number of animals in the former group. This beneficial effect is, however, overshadowed by additional deaths due to cardiac standstill, so that the overall survival rate was not significantly lowered. The dextro isomer of propranolol, which has practically no beta-blocking effect but has local anesthetic action, used in equiactive local anesthetic dose as that of the lower dose of *dl*-propranolol did not offer protection against

ventricular fibrillation. Ventricular fibrillation, in this study, was always fatal except in one dog in the MJ-1999 ED_{50} group. This dog went into ventricular fibrillation 90 seconds after coronary occlusion but recovered spontaneously in six seconds and survived the total duration of observation of 24 hours.

Since ventricular ectopic beats appearing in groups have a more serious prognostic implication than when they appear singly (Manning and Ahuja, 1969), the number of ventricular ectopic beats occurring in groups of four or more (ventricular tachycardia) were analyzed separately. It was found that treatment with this group of drugs significantly lowered the number of ectopic ventricular beats occurring in groups of four or more ($p < 0.01$ by analysis of variance) during the first five minutes following coronary occlusion. A closer look at the incidence of ventricular tachycardia shows that their number was significantly reduced by the lower dose of *dl*-propranolol, the higher dose of MJ-1999, the dextroisomer of propranolol alone and in combination with the lower dose of MJ-1999. The lower dose of MJ-1999 alone and the only dose of AY-21,011 used in our study did not significantly reduce the number of ventricular ectopic beats occurring in groups. The reduction of this arrhythmia by the higher dose of *dl*-propranolol fell just short of the level of significance ($p > 0.05 < 0.1$). From these results it seems quite possible that the membrane-effect of these drugs, in the doses used, may have some contribution in the reduction of ventricular tachycardia following coronary occlusion. The fact that there were more ectopic beats in the group treated with the higher dose of *dl*-propranolol than the lower dose is possibly related to increased conduction defect and

re-entry mechanism akin to that seen after quinidine overdose. Based on several deaths due to cardiac standstill in the AY-21,011-treated group, it would appear that the same mechanism is involved in the increased number of ventricular tachycardia in this group, although intrinsic sympathomimetic action of this drug remains a possibility. The number of ventricular ectopic beats appearing in groups of four or more were so few in the second and third five-minute periods that they were not subjected to statistical analysis.

The next order of serious arrhythmia is the ventricular ectopic beats occurring singly or in groups of three or less. The number of these ectopic beats ('premature ventricular beats') was not significantly lowered by any of the treatments in any of the three five-minute periods following acute coronary ligation. It thus appears that the more serious the form of arrhythmia, the better it is prevented by the beta-blocking drugs. When all ventricular ectopic beats without regard to their type are considered together, the difference in their incidence in the various groups is not statistically significant by overall analysis of variance. A closer scrutiny, however, reveals that their number is lesser in the groups treated with *dl*-propranolol ED_{50}, MJ-1999 ED_{100} and *d*-propranolol in equiactive local anesthetic dose of *dl*-propranolol.

It should now be asked to what extent may these findings, in healthy dogs undergoing sudden occlusion of a large coronary branch, be applicable to human disease in which the artery is presumably occluded more gradually. Besides, in man, coronary occlusion is not

uniformly observed at autopsy (Spiekerman, Brandenburg, Achor *et al.*, 1962) and fresh thrombi are not always found (Spain and Bradess, 1960). However, ventricular fibrillation has been shown to be the cause of sudden death following gradual occlusion of a coronary branch by ameroid constrictors in dogs (Vineberg *et al.*, 1960) and in farm pigs (Hurst, Morris, Zeft, Hackel and McIntosh, 1967). Also, Vineberg *et al.* (1960) have demonstrated that the total occlusion of a coronary artery was not necessary for the fatal outcome, that the critical point of coronary artery narrowing occurred when the diameters of two major arteries of the left ventricle were reduced by 40 percent or more and that 78 percent of animals died when both coronary branches to the left ventricle were narrowed but not totally occluded.

It has been estimated that about 23 percent of the patients sustaining a myocardial infarction die so suddenly that they never reach a hospital (Kannel *et al.*, 1961). This represents about 70 percent of patients dying from coronary artery disease (Kuller *et al.*, 1966). Ventricular fibrillation is most likely the mechanism of sudden death in these patients. This is supported by the findings with a mobile coronary care unit that the incidence of ventricular fibrillation is highest at the very inception of the ischemic episode (Pantridge and Geddes, 1967; Lawrie, 1969).

Lown (1970) has observed that when sudden death has occurred during electrocardiographic monitoring of patients with stable coronary artery disease, the mechanism has invariably been ventricular fibrillation. The association of clinical severity of myocardial infarction and of ventricular arrhythmia with urinary excretion of

catecholamines has been demonstrated in 55 patients by Jewitt *et al.* (1969).

Routine use of beta-blocking drugs, despite an earlier enthusiastic report (Snow, 1965) has not generally been helpful in patients already admitted to hospital (Balcon *et al.*, 1967). The real problem of sudden death from coronary artery disease resides not in the hospital but in the community. If the high mortality rate from this disease is to be reduced, it will most probably be by identifying and treating the individuals with a high risk for sudden death. The concept of "precoronary care" has evolved in order to tackle this serious problem (Lown, Klein and Hershberg, 1969; Lown and Ruberman, 1970). It is important to note that ventricular fibrillation is not necessarily associated with large infarcts and that when successfully resuscitated, the life expectancy of patients after resuscitation is not significantly lower than in patients with similar infarction who do not experience this arrhythmia (Geddes, Adgey and Pantridge, 1967; Stannard and Sloman, 1969). So prevention of ventricular fibrillation in the earliest phase of myocardial infarction seems to be the crucial factor in the prevention of abrupt death from coronary artery disease.

Although these deaths are sudden with acute ischemic episode, they are not always without warning. More than half of sudden coronary artery disease deaths occur in a definable group having either previous myocardial infarction, angina pectoris or known "risk factors" for arteriosclerotic heart disease. Solomon, Edwards and Killip (1969) have reported that 65 percent of patients reaching a coronary care unit had significant symptoms of heart disease during

the month prior to hospitalization for acute myocardial infarction. Kuller, Lilienfeld and Fisher (1966) and Kuller (1969) found that 23 percent of coronary victims with sudden death had seen a physician one week prior to death. Lown and Ruberman (1970) reported that Tibblin of Goteborg, Sweden noted that one-third of patients dying suddenly had consulted their physicians within two days and one-half within two weeks before death. The chief complaints were changing anginal pattern and unusual fatigue. This group of patients thus lend themselves to some sort of prophylactic measures against ventricular fibrillation.

Our study justifies an evaluation of the beta-adrenergic receptor blocking drugs in carefully selected high-risk individuals. The total population at risk is far too large and heterogeneous to be dealt with clinically. Lown and Ruberman (1970) have attempted to classify the high-risk population into subgroups according to the magnitude of the risk of sudden death. Although more research in case identification is needed, it is now known that the incidence of sudden death is inordinately high in patients who recover from myocardial infarction (Weinblatt *et al.*, 1968) and in individuals exhibiting ventricular premature beats with or without coronary artery disease (Chiang, Perlman, Ostrander and Epstein, 1969 ; Hinkle, Carver and Stevens, 1969). With the advent of the beta-blocking drugs, it is clearly time for a carefully controlled study in a selected group of such high-risk individuals.

A retrospective and a prospective study could easily be

undertaken without any delay and without subjecting any new population to any new drug. Many patients with angina pectoris are now already taking *dl*-propranolol. It would be worthwhile to find out the incidence of sudden death in patients receiving *dl*-propranolol compared to that in similar patients who are not being treated by this drug. Nevertheless, other concomitant drug therapy might be a complicating factor in the interpretation of the results obtained.

From the present study, it is not possible to state which of these drugs may be the most useful agent in clinical trial. In our investigation, the early mortality rate of 17 percent in the groups treated with MJ-1999 (0.2 mg/kg) plus *d*-propranolol (0.0723 mg/kg) and with MJ-1999 (3.2 mg/kg) is not significantly different from the mortality rates of 24 percent, 28 percent and 31 percent in the groups receiving *dl*-propranolol (0.1 mg/kg), MJ-1999 (0.2 mg/kg) and AY-21,011 (1.4 mg/kg), respectively ($p > 0.75$). However, from our study and from those by others, certain guidelines are apparent regarding the use of the currently available beta-blocking drugs. Prominent intrinsic sympathomimetic activity may limit the use of certain drugs, e.g., dichloroisoproterenol (Blinks, 1967; Dunlop and Shanks, 1968). Potential carcinogenic effect has led to the withdrawal of pronethalol from clinical use (Paget, 1963). Whatever may be the fundamental mechanism, *dl*-propranolol seems to have more direct myocardial depressant action and, in higher doses, causes more conduction defects following experimental coronary occlusion than does MJ-1999. Cardio-selective beta-blockade by

AY-21,011 has certain theoretical advantages over non-selective beta-blockade in clinical situations. The absence of a significant fall in blood pressure and a significant rise in peripheral vascular resistance by up to 25 mg of the drug, and the absence of a significant fall in cardiac output by doses up to 15 mg of AY-21,011 injected intravenously in patients with ischemic heart disease are of great clinical importance. Given in small doses, this drug has a negative chronotropic and a positive inotropic effect (Sowton *et al.*, 1968). AY-21,011 has been claimed to have a greater margin of safety than *dl*-propranolol in patients with impaired myocardial function (Gibson *et al.*, 1968). While *dl*-propranolol significantly increases the airway resistance in normal as well as asthmatic patients, AY-21,011 has very little effect on airway resistance even in patients with bronchial asthma (Macdonald, Ingram and McNeill, 1967; Macdonald and McNeill, 1968). Moreover, AY-21,011 blocks the effects of sympatho-adrenal discharge on the heart and, at the same time, augments coronary flow (Bussmann, Rauh and Krayenbuehl, 1970). This is in sharp contrast to the effects of non-selective beta-blockers, e.g., *dl*-propranolol (Nayler, McInnes, Swann, Carson and Lowe, 1967). The optimism regarding the pharmacological properties of AY-21,011 should, however, be a cautious one. Bussmann *et al.* (1970) reported progressive impairment of left ventricular contractility with increasing doses of 2-8 mg/kg of this drug in closed-chest dogs. Our study indicates that the stress of acute myocardial infarction may lead to serious depression of the conduction system if this drug is given in a relatively high dose. The doses in which these agents are administered is very important

in the final outcome. Increasing the dose of some of these compounds does not necessarily cause a further decrease in the mortality and may even increase it.

SUMMARY

Arteriosclerotic heart disease exists in the North American continent in epidemic proportions. Most of the deaths due to this disease are sudden, unexpected and occur during the earliest phase of an acute ischemic episode. These sudden deaths are thought to be due to ventricular fibrillation. Clinical and experimental evidence suggests that the sympathetic nerves to the heart and the catecholamines play a very important role in the onset of ventricular fibrillation soon after coronary occlusion. Interference with the sympathetic nerve supply to the heart and depletion of catecholamines have previously been shown to decrease the incidence of ventricular fibrillation in experimental coronary occlusion in animals.

The present investigation is concerned with the influence of various beta-receptor blocking drugs in experimental coronary occlusion in conscious dogs. This was carried out in 141 fully conscious mongrel dogs by tying the most proximal part of the circumflex branch of the left coronary artery. Direct arterial blood pressure and electrocardiogram were monitored continuously for one to two hours or until death. The dogs were randomly allocated to eight experimental groups as follows:

Group 1 received 2-3 ml normal saline and served as control (25 dogs)

Group 2 received *dl*-propranolol, 0.1 mg/kg (25 dogs)

Group 3 received *dl*-propranolol, 1.0 mg/kg (10 dogs)

Group 4 received MJ-1999, 0.2 mg/kg (25 dogs)

Group 5 received MJ-1999, 3.2 mg/kg (12 dogs)

Group 6 received *d*-propranolol, 0.0723 mg/kg (16 dogs)

Group 7 received *d*-propranolol, 0.0723 mg/kg + MJ-1999, 0.2 mg/kg (12 dogs)

Group 8 received AY-21,011 , 1.4 mg/kg (16 dogs)

The drug or saline was administered intravenously five minutes before coronary occlusion. The doses of these drugs were selected by preliminary experiments whereby their ability to antagonize the chronotropic effect of isoproterenol (1.5 µg/kg) was determined. Thus the doses of *dl*-propranolol, MJ-1999 and AY-21,011 used in Groups 2, 4 and 8, respectively, were equiactive in that they all caused a 50 percent reduction in the tachycardia provoked by isoproterenol challenge. Likewise, the doses of *dl*-propranolol and MJ-1999 in Groups 3 and 5, respectively, are also equiactive to each other causing nearly 100 percent blockade of isoproterenol-induced tachycardia. The dose of *d*-propranolol, used alone in Group 6 and in combination with the ED_{50} dose of MJ-1999 in Group 7, has been shown to have equal local anesthetic effect as that of the ED_{50} dose of *dl*-propranolol used in Group 2.

The two-hour mortality rates in Groups 2, 4, 5, 7 and 8 were 24, 28, 17, 17 and 31 percent, respectively, and were significantly lower than the 72 percent mortality rate in the control group ($p < 0.025$). The mortality rates in Groups 3 and 6 were 70 and 69 percent, respectively, and were not significantly different from that of the control group. All deaths were due to ventricular fibrillation except for one dog in Group 2, three dogs in Group 3 and two dogs in Group 8. These latter dogs died with cardiac standstill. Since

dl-propranolol possesses both beta-blocking effect and local anesthetic action and since MJ-1999 is a pure beta-blocking agent without any local anesthetic effect, it is concluded that the beneficial effect of these agents in the doses used, in experimental coronary occlusion, is primarily due to their beta-blocking property and not due to their local anesthetic effect. This view is further supported by the fact that *d*-propranolol in a dose having the same local anesthetic effect as the ED_{50} dose of *dl*-propranolol did not offer similar protection against ventricular fibrillation. Since the cardioselective beta-blocking agent AY-21,011 significantly reduced the mortality rate, it is concluded that the blockade of the beta-receptors in the heart alone is sufficient for the beneficial effect; the lack of local anesthetic effect of AY-21,011 further substantiates the view that the beneficial effect of these drugs, in the doses used, is mainly due to beta-blockade.

The dose in which these drugs are administered is very important. Thus the antifibrillatory effect of *dl*-propranolol in the high dose was overshadowed by deaths due to cardiac standstill. For reasons discussed, the dose of AY-21,011, used in this study, was most likely higher than the ED_{50} doses of *dl*-propranolol and MJ-1999; an increased incidence of conduction defects was noted with this dose.

Analysis of arrhythmias revealed that the more serious the arrhythmia following coronary occlusion, the better was it controlled by beta-blockade. The protection against ventricular fibrillation was mainly due to beta-blockade. Both the beta-blocking and local anesthetic effects of these drugs reduced the incidence of ventricular

tachycardia. Ventricular premature beats, appearing singly or in runs of three or less, were not modified by any of these agents.

The heart rate was decreased and the arterial blood pressure was raised by these agents, but the magnitude of the change was small and was not always significant. Except for the group treated with AY-21,011, the PR interval was significantly prolonged by these agents, including *d*-propranolol. Following acute coronary occlusion, there was a rise in the heart rate and a fall in the arterial blood pressure. These changes were not significantly altered by the drugs used in this study.

If the high rate of sudden death from coronary artery disease is to be controlled in the near future, it has to be by the identification and prophylactic treatment of individuals with high risk for sudden death from arteriosclerotic heart disease. The present study justifies an investigation of these agents in clinical practice and forms a basis for a rational approach towards therapy.

CONCLUSIONS

1. The beta-adrenergic receptor blocking drugs, *dl*-propranolol, MJ-1999 and AY-21,011, in appropriate doses, significantly reduce the mortality rate caused by experimental coronary occlusion in conscious dogs.

2. It is the beta-blocking property of these agents, rather than their local anesthetic effect, that is primarily responsible for the reduction of mortality rate with this group of drugs in the doses used.

3. Great caution is needed in the selection of suitable doses of these drugs. Although a particular dose of these agents may not cause any serious depression of impulse formation and/or conduction in normal dogs, serious cardiodepressive effects, sometimes to the point of cardiac standstill, occur when the stress of coronary occlusion is superimposed. Thus, increasing the dose of some compounds does not necessarily cause a further decrease in mortality and may even increase it (e.g., *dl*-propranolol).

4. The more serious the arrhythmia, following acute coronary occlusion, the better is it prevented by the beta-blocking drugs; thus ventricular fibrillation, ventricular tachycardia and isolated premature ventricular beats were influenced by these agents in decreasing order.

5. The local anesthetic effect of the doses of these agents used

decreases the number of ectopic ventricular beats appearing in runs of four or more (i.e., ventricular tachycardia).

6. In dogs the heart rate is decreased and arterial blood pressure is increased by the beta-blocking drugs; however, these changes are not always significant.

7. Except for AY-21,011, the PR interval is significantly increased by all other drugs studied, including *d*-propranolol.

8. The rise in the heart rate and the fall in the arterial blood pressure immediately following acute coronary occlusion are not significantly modified by the doses of these agents used.

9. This investigation forms a rational basis for the use of beta-adrenergic receptor blocking drugs in patients with a predisposition to sudden death associated with coronary artery disease.

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APPENDIX I

Data used for the calculation of regression equations for *dl*-propranolol-, MJ-1999-, and AY-21,011-blockade of isoproterenol-induced tachycardia.

APPENDIX I

[X = log dose, Y = mean percentage block]

A. The blockade of isoproterenol-induced tachycardia by *dl*-propranolol in conscious dogs (dose of isoproterenol = 1.5 µg/kg).

Dose in mg/kg	Percentage Blockade *	Mean
0.05	33,33	33
0.10	55,51,58,35	49.75
0.20	74,79,85,51	72.25
0.40	79,74,91,92	84
0.60	85	85
0.80	97,92	94.5
1.00	95,100,93,94,93,100	95.8

B. The blockade of isoproterenol-induced tachycardia by MJ-1999 in conscious dogs (dose of isoproterenol = 1.5 µg/kg).

Dose in mg/kg	Percentage Blockade *	Mean
0.1	32,52,34	39
0.2	38,59,56	51
0.4	67,65,63	65
1.6	82,94,89	88
2.4	90,94,92	92

C. The blockade of isoproterenol-induced tachycardia by AY-21,011 in conscious dogs pre-treated with 0.2 mg/kg atropine (dose of isoproterenol = 1.5 µg/kg).

Dose in mg/kg	Percentage Blockade *	Mean
0.4	36,48,15	33
1.6	56,51,47	51
6.4	69,57,85	70

* Values represent percentage blockade five minutes after administration of the appropriate blocking agent, being the same interval between drug administration and coronary ligation in the subsequent experiments.

APPENDIX II

Time-course of the mean heart rate, systolic blood pressure and diastolic blood pressure from before coronary occlusion to the end of one to two hours follow-up period.

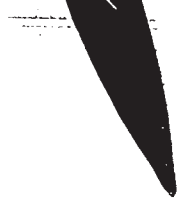
Time-course of the heart rate (Mean ± S.E.M.)

(A = Control, B = five minutes after normal saline or drug. The times from the moment of coronary occlusion are indicated.)

TIME-COURSE OF HEART RATE

MEAN ± S.E.M.

Group	A	B	1/2 Min	1 Min	1 1/2 Min	2 Min	3 Min	4 Min	5 Min	10 Min	15 Min	30 Min	45 Min	60 Min	90 Min	120 Min
1.	121.9 ± 5.0	123.2 ± 5.0	178.6 ± 8.5	181.6 ± 12.6	158.4 ± 12.9	145.0 ± 9.2	140.3 ± 12.8	155.1 ± 18.2	167.0 ± 23.3	154.4 ± 20.0	129.6 ± 12.9	119.0 ± 19.4	126.1 ± 19.4	110.0 ± 16.5	108.0 ± 17.9	109.2 ± 15.2
2.	107.4 ± 4.8	99.6 ± 4.6	146.9 ± 5.7	147.5 ± 5.8	139.6 ± 6.4	132.0 ± 6.1	131.9 ± 9.9	128.7 ± 6.1	123.5 ± 9.4	125.4 ± 8.1	126.5 ± 7.2	131.1 ± 7.7	129.2 ± 6.8	125.4 ± 11.8	116.3 ± 10.2	123.6 ± 8.6
3.	122.5 ± 7.3	110.9 ± 5.6	141.7 ± 10.6	141.3 ± 8.1	140.7 ± 6.3	137.5 ± 6.4	141.6 ± 6.7	147.3 ± 8.7	147.6 ± 8.0	127.5 ± 13.2	123.8 ± 16.7	148.3 ± 9.5	137.0 ± 5.9	137.0 ± 5.9	129.0 ± 5.7	131.7 ± 7.6
4.	118.7 ± 2.7	112.8 ± 2.8	174.6 ± 6.2	165.5 ± 6.0	161.0 ± 6.2	163.1 ± 6.4	157.2 ± 6.7	153.1 ± 6.9	153.1 ± 6.2	160.9 ± 8.4	165.9 ± 11.1	163.4 ± 12.0	163.9 ± 10.3	159.4 ± 11.1	154.2 ± 9.2	142.1 ± 10.9
5.	95.4 ± 6.5	89.5 ± 4.4	128.3 ± 4.7	122.5 ± 5.4	118.3 ± 5.0	114.0 ± 4.9	117.5 ± 5.0	116.3 ± 5.1	116.9 ± 5.6	121.0 ± 4.1	123.5 ± 3.4	129.0 ± 4.8	131.0 ± 5.4	131.2 ± 5.7		
6.	117.3 ± 9.5	112.3 ± 9.5	193.1 ± 24.0	171.7 ± 12.7	146.7 ± 14.0	148.9 ± 14.4	145.6 ± 16.5	154.7 ± 15.7	162.3 ± 14.2	148.3 ± 15.4	154.3 ± 15.8	157.4 ± 8.0	157.2 ± 12.1	154.8 ± 15.5		
7.	114.4 ± 5.0	111.0 ± 4.3	165.3 ± 7.6	160.7 ± 10.8	144.0 ± 7.6	135.2 ± 10.0	144.0 ± 9.9	158.9 ± 17.9	161.2 ± 18.0	159.8 ± 13.8	159.6 ± 13.5	150.7 ± 6.4	147.7 ± 7.9	147.9 ± 7.1		
8.	109.6 ± 7.1	105.8 ± 7.2	160.8 ± 9.2	182.2 ± 23.9	138.6 ± 10.2	141.9 ± 9.0	141.1 ± 13.8	137.9 ± 11.7	140.0 ± 10.6	158.6 ± 12.8	163.5 ± 15.4	156.9 ± 16.3	158.5 ± 18.2	156.0 ± 17.4		



Time-course of systolic blood pressure (Mean ± S.E.M.)

(A = Control, B = Five minutes after normal saline or drug. The times from the moment of coronary occlusion are indicated.)

TIME-COURSE OF SYSTOLIC BLOOD PRESSURE

MEAN ± S.E.M.

Group	A	B	1/2 Min	1 Min	1 1/2 Min	2 Min	3 Min	4 Min	5 Min	10 Min	15 Min	30 Min	45 Min	60 Min	90 Min	120 Min
1.	153.4 ± 4.8	154.3 ± 4.3	133.2 ± 8.0	118.7 ± 8.4	117.1 ± 11.2	118.7 ± 8.5	122.3 ± 7.3	127.3 ± 5.9	128.4 ± 8.0	132.4 ± 5.9	129.7 ± 6.7	129.4 ± 6.9	125.4 ± 6.7	133.7 ± 6.3	122.3 ± 4.6	121.3 ± 6.2
2.	154.9 ± 4.0	164.8 ± 4.2	136.0 ± 6.6	124.9 ± 7.0	120.8 ± 7.9	122.7 ± 8.8	121.7 ± 8.2	129.2 ± 5.7	138.7 ± 6.6	134.2 ± 5.8	135.0 ± 5.3	138.3 ± 4.8	140.6 ± 5.1	134.2 ± 6.2	134.2 ± 4.4	135.2 ± 4.5
3.	155.0 ± 6.7	161.4 ± 5.6	124.4 ± 11.0	118.2 ± 11.9	113.8 ± 11.9	113.2 ± 13.5	123.8 ± 10.8	134.6 ± 9.6	137.1 ± 9.0	126.6 ± 15.2	126.5 ± 24.8	157.3 ± 22.7	148.0 ± 17.0	145.3 ± 14.2	131.1 ± 13.4	120.0 ± 6.8
4.	147.1 ± 3.7	151.8 ± 3.9	139.9 ± 4.0	131.6 ± 5.7	128.9 ± 5.8	128.6 ± 5.0	128.9 ± 5.2	128.7 ± 4.5	131.1 ± 4.6	135.9 ± 4.6	138.5 ± 4.9	136.7 ± 6.1	141.9 ± 4.4	143.3 ± 4.9	139.2 ± 6.5	136.5 ± 6.3
5.	158.5 ± 6.0	161.0 ± 7.4	147.5 ± 10.3	145.8 ± 9.5	140.5 ± 8.1	131.3 ± 8.0	133.1 ± 8.1	137.8 ± 7.7	140.4 ± 7.8	145.5 ± 7.0	149.3 ± 7.0	147.8 ± 5.3	154.2 ± 6.7	150.2 ± 6.3		
6.	164.0 ± 6.7	168.5 ± 8.1	152.9 ± 7.2	132.6 ± 9.3	126.7 ± 13.2	132.0 ± 14.8	138.9 ± 12.1	143.4 ± 10.6	139.4 ± 9.2	145.0 ± 14.3	151.0 ± 14.1	152.0 ± 10.9	155.2 ± 10.4	152.0 ± 10.7		
7.	164.3 ± 5.5	170.0 ± 6.8	136.3 ± 9.4	112.4 ± 6.5	116.0 ± 5.8	116.6 ± 7.8	119.4 ± 6.5	120.2 ± 6.7	119.2 ± 6.0	130.0 ± 5.6	131.6 ± 4.8	136.8 ± 4.7	137.0 ± 5.6	137.6 ± 5.6		
8.	162.1 ± 5.6	168.6 ± 5.8	149.1 ± 6.6	133.1 ± 9.4	125.7 ± 13.3	138.1 ± 10.7	139.8 ± 13.2	152.0 ± 6.1	152.2 ± 6.6	153.3 ± 6.2	154.4 ± 6.4	152.7 ± 7.7	150.9 ± 9.9	153.1 ± 7.5		

Time-course of diastolic blood pressure (Mean ± S.E.M.)

(A = Control, B = Five minutes after normal saline or drug. The times from the moment of coronary occlusion are indicated.)

TIME-COURSE OF DIASTOLIC BLOOD PRESSURE

MEAN ± S.E.M.

Group	A	B	1/2 Min	1 Min	1 1/2 Min	2 Min	3 Min	4 Min	5 Min	10 Min	15 Min	30 Min	45 Min	60 Min	90 Min	120 Min
1.	91.0 ± 3.4	92.0 ± 3.2	86.6 ± 6.0	76.5 ± 6.5	73.6 ± 8.1	71.5 ± 5.8	78.0 ± 4.9	81.5 ± 5.0	86.3 ± 4.3	83.2 ± 4.4	81.1 ± 4.3	75.4 ± 6.6	73.7 ± 6.3	74.0 ± 5.9	63.0 ± 5.3	61.3 ± 6.7
2.	92.6 ± 2.9	102.7 ± 3.3	94.4 ± 5.0	84.6 ± 6.0	83.4 ± 6.8	82.9 ± 7.2	83.9 ± 6.3	88.7 ± 5.2	97.6 ± 4.7	93.9 ± 4.9	95.8 ± 4.2	94.8 ± 4.3	94.5 ± 4.0	91.3 ± 4.6	87.5 ± 5.3	90.7 ± 5.2
3.	91.0 ± 3.1	97.4 ± 3.8	81.4 ± 10.0	79.4 ± 9.8	76.8 ± 9.6	75.8 ± 10.1	84.0 ± 8.2	94.3 ± 7.1	97.4 ± 6.3	84.0 ± 11.3	84.5 ± 18.5	110.0 ± 17.7	102.7 ± 13.9	98.7 ± 11.4	87.3 ± 9.5	78.0 ± 2.8
4.	83.4 ± 2.6	89.4 ± 2.8	90.3 ± 3.5	83.8 ± 5.2	84.0 ± 5.0	87.5 ± 4.7	87.3 ± 5.1	88.4 ± 4.7	90.0 ± 4.6	95.2 ± 4.5	96.7 ± 5.1	93.6 ± 5.5	96.4 ± 3.7	95.0 ± 4.3	92.0 ± 5.3	86.5 ± 6.5
5.	86.3 ± 3.0	92.7 ± 5.0	99.8 ± 9.0	99.7 ± 9.4	94.2 ± 8.1	86.9 ± 7.1	88.9 ± 8.0	94.7 ± 7.4	96.7 ± 7.4	101.3 ± 5.5	108.9 ± 5.9	107.4 ± 3.6	112.0 ± 5.3	106.7 ± 6.2		
6.	95.5 ± 4.5	95.8 ± 3.9	103.0 ± 6.5	90.2 ± 6.7	82.7 ± 10.0	85.5 ± 10.7	90.3 ± 9.4	96.0 ± 8.0	94.9 ± 6.5	94.0 ± 9.0	98.0 ± 8.1	99.6 ± 7.6	102.4 ± 7.0	95.2 ± 6.2		
7.	95.8 ± 4.2	106.2 ± 5.7	85.7 ± 7.0	70.2 ± 6.4	76.8 ± 6.8	75.2 ± 8.5	79.1 ± 7.5	81.4 ± 7.1	81.0 ± 6.9	90.4 ± 4.6	91.4 ± 4.7	96.0 ± 4.4	94.0 ± 5.5	93.6 ± 6.2		
8.	89.5 ± 3.4	97.1 ± 3.3	93.9 ± 4.8	84.4 ± 7.7	74.6 ± 8.8	89.2 ± 8.2	95.7 ± 9.6	101.8 ± 6.0	102.4 ± 5.2	105.3 ± 4.4	105.5 ± 3.9	101.5 ± 4.6	95.3 ± 6.6	97.3 ± 5.0		