Controlled Myocardial Injury Produced by a Hypothermal Method

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Following a review of experimental methods used for the production of necrosis or inactivation of the myocardium, a new method employing low temperatures is described. Lesions, with complete necrosis of muscle cells, can be controlled as to size and can be selectively placed to involve any of the four cardiac chambers and the interventricular septum. This facilitates attempts to study chronic myocardial insufficiency in terms of location and size of damaged muscle. Also, it has been possible to produce disturbances in conduction by production of small interventricular septal lesions. Refined electrocardiographic analysis of the magnitude and location of ventricular lesions has not been possible.

The experimental production of anatomic and functional myocardial injury has become a useful technic in physiologic, pathologic and electrophysiologic studies of the heart. Necrosis or inactivation of portions of the myocardium has been produced by various methods. Methods used previously will be discussed and a new hypothermal method, having certain advantages, will be described. All procedures discussed are modifications of one of three methods for the production of necrosis or alteration of function of myocardium, namely: ischemic necrosis or injury, necrosis or alteration of function with physical agents and injury or alteration of function with chemical agents.

Historical Review

Experimental myocardial infarction was first attempted by Panum in 1862 by the injection of a mixture of oil, wax, tallow and lampblack into the aortas of dogs. His results were not conclusive. Samuelson in 1881 and later Fenoglio and Drogouil in 1888 described the production of myocardial necrosis, in acute experiments in dogs, by the ligation of major branches of the left coronary artery. In acute and chronic experiments, Kolster in 1893 ligated small branches of the anterior descending branch of the left coronary artery. Animals were kept for as long as 17 months after the production of lesions. Porter, in 1896, reported a large series of acute experiments in which he ligated major branches of the left coronary artery, the right coronary artery and branches to the interventricular septum. In some experiments, he occluded orifices of the coronary arteries with a curved glass rod which was inserted into the aorta and sinus of Valsalva through a slit in the innominate artery.

Necrosis of the myocardium with a sclerosing chemical agent was first produced by Lohman in 1908. This investigator used sponges saturated with formalin to kill right atrial and adjacent muscle cells in rabbits. He was able to inactivate the sinoauricular node without hemorrhage and without interference with the flow of blood through its normal channels. Other early investigators who reported the production of myocardial necrosis in experimental animals are: Miller and Matthews (1909) who studied the value of digitalis and strophanthin after ligation of the circumflex branch of the left coronary artery, Kahn (1911) who first observed a negative T wave in the electrocardiogram following the ligation of coronary arteries, and Smith (1918) who reported electrocardiographic and postmortem studies on

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66 dogs after ligation of the right coronary artery and branches of the left coronary artery.

**Production of Ischemic Necrosis or Injury**

Ligation of Large Coronary Vessels

Ischemic necrosis of large areas of the heart by permanent and complete ligation of the right coronary artery or a major branch of the left coronary artery of dogs has been used by many investigators.\(^8\)\(^-\)\(^22\) Temporary occlusion of major branches of the left coronary artery of dogs has been reported.\(^3\),\(^ 23\)\(^-\)\(^27\) This procedure has been used for electrocardiographic studies of early changes in the R-ST segment and T wave in epicardial leads.\(^24\),\(^ 27\) Bronson\(^24\) studied hearts of dogs thirty days after periods of ischemia ranging from 30 minutes to 2 hours. Ischemic periods of 30 minutes produced small focal areas of fibrosis; periods of 2 hours produced large fibrous scars similar to those seen after coronary thrombosis in man. Partial occlusion of coronary arteries was produced in dogs by Blumgart and co-workers\(^28\) by ligating the vessel with a wire, having a known diameter, within the ligature; after ligation the wire was removed. Myocardial necrosis following the ligation of the coronary sinus and other cardiac veins has been reported by Robertson.\(^29\)

Ligation of the anterior descending branch of the left coronary artery of dogs has been used in the evaluation of drugs used clinically following myocardial infarction.\(^30\)\(^-\)\(^34\) This technic was used to study the effect of a sympathectomy on the size of myocardial infarcts.\(^35\),\(^ 36\) A sympathectomy did not reduce the size of infarcts.

**Ligation of Small Arteries**

Ligation of the right coronary artery or a major branch of the left coronary artery of dogs often precipitates fatal ventricular fibrillation or death within 24 hours. Ligation of small secondary arteries\(^37\)\(^-\)\(^43\) has obviated this complication and has been useful for the production of small myocardial infarcts for electrocardiographic studies.

**Ligation of Nutrient Arteries to Certain Regions**

Necrosis of certain portions or structures of the heart has been produced by ligation of their nutrient arteries. Cushing and associates\(^44\) ligated all arteries to the right and/or left atrium in 22 dogs. Necrosis of atrial myocardium, particularly subendocardial fibers, was not complete. Electrocardiographic changes were absent in 12 of the animals, transient in 4 and permanent in 6. In acute experiments, Kahn\(^4\) ligated the interventricular branch of the left coronary artery and demonstrated right bundle branch block within 5 minutes. Removal of the ligature resulted in the disappearance of abnormal conduction. Kisch,\(^4\) using a perfused, isolated heart, reported the production of complete atioventricular block following ligation of the left coronary artery. Normal conduction was restored by opening the left coronary artery and occluding the right coronary artery. Lauterbach\(^4\) reported the production of right or left bundle branch block following the ligation of the interventricular septal artery. Barton and Greenwood\(^47\) were unable to demonstrate a permanent disturbance in cardiac conduction following ligation of the septal artery in spite of gross and microscopic evidence of large septal infarcts. In one animal there was a temporary complete atroventricular dissociation one day after ligation of the septal artery. This disappeared within 48 hours.

Wilson\(^4\) ligated the septal artery in 3 dogs. Large septal infarcts were described. Right bundle branch block was observed in all animals. One experiment was an acute experiment; two were terminated after 48 hours.

In attempts to produce failure of the right heart, Starr\(^49\) ligated all branches of the right coronary artery leading to the right ventricle. A postmortem examination was reported as showing only partial infarction of the right ventricle. Landis and co-workers,\(^50\) in an interesting study of failure of the right heart, ligated the right coronary artery in order to produce decompensation of the right ventricle. Since his experiments were acute and postmortem studies were not reported, the degree of necrosis of right ventricular myocardium is not known.

**Embolic Occlusion of Vessels**

The injection of particulate matter for embolic occlusion of coronary arteries has been
used by a number of investigators including Hamburger and associates, Herman and Decherd, and Roos and Smith. Lycopodium spores in suspension were injected by Hamburger and co-workers. Herman and Decherd described an ingenious method for the production of myocardial necrosis with a minimum of surgical trauma. Myocardial infarction was produced by instilling a drop of mercury into a coronary artery through a cardiac catheter that had been inserted through the carotid artery and into the sinus of Valsalva.

Roos and Smith described the production of acute cardiac failure in 15 to 35 minutes in dogs by injection of a suspension of starch in saline into the left ventricle. The aorta was occluded just above the aortic cusps during and briefly after the injection of the suspension of starch. The animals died within 10 minutes after evidences of cardiac failure were observed. Embolization of the systemic circulation was not ruled out in their experiments. They reviewed other work on experimental heart failure.

**Experimental Coronary Thrombosis**

The production of coronary thrombosis, experimentally, has been reported by Hall, Ettinger and Banting. They reported degenerative changes in coronary arteries of old and middle aged dogs after daily injections of acetylcholine iodide or acetylcholine bromide for one to eight months. Thrombosis of coronary arteries was observed in 2 old dogs receiving the drug. Control studies and the number of animals used in the experiment were not mentioned. This work was repeated by Horswell who used larger doses of acetylcholine. Degenerative changes in the myocardium and coronary arteries were not found by this investigator. Prolonged electrical stimulation of the vagus nerve (Ettinger and co-workers and Manning and associates) produced no changes in coronary arteries and controversial changes in the myocardium. Vagal stimulation produced by daily injections of extracts of the posterior lobe of the pituitary gland for three months to four and one-half years failed to produce coronary thrombosis or myocardial degeneration (Ettinger and associates). The above evidence suggests that stimulation of parasympathetic fibers to the heart and the administration of parasympatheticomimetic drugs probably do not produce significant changes in the myocardium and coronary arteries.

**Necrosis or Alteration of Function of Myocardium Produced by Physical Agents**

**Hyperthermal Injury of Myocardium**

Electrocautery has been used for the production of myocardial necrosis by a number of investigators. Thomas and Harrison and Starr inactivated portions of hearts with heated metal. Cushing and co-workers reported atrial rupture within a few minutes after cautery of the atria of dogs. In studies on cats, Crawford found that complete penetration of the wall of the heart with electrocautery resulted in myocardial rupture within a short time. Most of the above investigators found hyperthermal injury satisfactory for only acute experiments unless precautions were taken to avoid complete penetration of the wall of the heart. Thomas and Harrison produced hyperthermal injury of the left ventricle of rats for chronic experiments but a number of their animals died from rupture of the heart.

**Injury of Myocardium from X-Ray**

Irradiation from radon seeds (3 to 10 mc.) in a small glass capillary tube, has been used for the production of localized myocardial injury by Haney and associates. The capillary glass tubes, containing radon seeds, were inserted into various locations in the ventricular myocardium of dogs. Lesions produced by this method had variable diameters ranging from 1 to 3 cm. It is of interest that frequently their animals died from a ruptured myocardium even after electrocardiograms indicated a healing myocardial infarct. Microscopic studies of the lesions were not reported.

**Hypothermal Injury of Myocardium**

Eppinger and Rothberger reported freezing local areas of the myocardium with an ethyl chloride spray. Smith briefly described the
application of test tubes, filled with hot and cold liquids, to the myocardium.

Hoff and Nahum described the use of small (1 cm. diameter) metal thermodes cooled by circulating warm or cold water. The thermodes were placed on specific locations on the epicardium and endocardium. The endocardial thermode was attached to a 10 cm. plastic tube and apparently was inserted through the great vessels. The method of insertion of the endocardial thermode is not described. This technic was used in a study of the genesis of T-wave changes.

Myocardial Injury from Blows

Myocardial necrosis has been produced experimentally by striking the heart and the thorax with various instruments. These studies are related to traumatic injuries of the heart and will not be discussed further.

Surgical Myocardial Injury

Murray has described surgical resection of infarcted left ventricular myocardium produced by ligation of the anterior descending branch of the left coronary artery. Resection of the infarcted myocardium resulted in a decreased incidence of fatal ventricular fibrillation following ligation of the anterior descending branch. Surgical extirpation of infarcted myocardium, which diluted during systole, resulted in an increased cardiac output and elevation of the blood pressure.

Barger, Richardson and Roe have recently described a surgical technic for the production of chronic right-sided cardiac failure in dogs. Chronic right-sided cardiac failure occurred only in animals with surgically induced insufficiency of the tricuspid valve and stenosis of the pulmonary artery.

Injury or Alteration of Function of Myocardium Produced with Chemical Agents

Production of Myocardial Injury with Necrotizing Chemical Agents

It has been mentioned previously that Lohman in 1908 first described the use of a necrotizing chemical for the production of myocardial necrosis. Hering in 1909 also described the use of sponges saturated with formalin for the production of myocardial necrosis. Solutions of mercuric chloride (5 per cent) and silver nitrate (20 per cent) were injected into the myocardium by Eppinger and Rothberger. They reported that the injection of silver nitrate (20 per cent) produced localized lesions which could be well outlined grossly and microscopically. The injection of mercuric chloride was not satisfactory. Smith found that the injection of solutions of mercuric chloride and nitric acid was unsatisfactory because limiting the effects to definite portions of the myocardium was impossible. Otto, who injected a 95 per cent solution of alcohol, also encountered difficulty in limiting lesions to certain areas. Others have described the use of necrotizing chemical agents for the production of myocardial necrosis in acute experiments. Cushing and co-workers, using dogs, found that the application of solutions of phenol to the atrium resulted in death within one hour. Death was believed to have been due to the systemic, toxic effect of phenol. In general, the above investigators found necrotizing chemical agents useful for acute experiments. When chronic experiments were performed, ligation of nutrient vessels was usually the method used.

Production of Pericarditis with Chemical Agents

Although the technics described by Beck, Armstrong and Fishman and associates are not methods for the production of myocardial injury, they are excellent methods for the production of right-sided chronic passive congestion. Beck and Armstrong injected irritating chemical solutions into the pericardial cavity. Fishman and co-workers inserted cellophane into the pericardial sac. Animals subjected to the above procedures developed progressive increases in venous pressure with chronic passive congestion of the liver, ascites and edema.

Chemical Agents that Disturb Cardiac Electrophysiology

During recent years the application of blotting paper saturated with potassium chloride (fifth molar) and other agents to the surface of the heart has been used for the
production of temporary disturbances in the electrophysiologic behavior of the surface of the heart.\textsuperscript{79-90} Calcium chloride, strophanthin and ouabain produce similar effects. In 1920, Hoffman\textsuperscript{79} described the use of blotting paper saturated with potassium chloride for the production of blocks in the atrioventricular conduction mechanism in isolated frogs' hearts. Later, Kisch\textsuperscript{80,81} described the use of this technic for the production of electrocardiographic changes (elevation of the S-T segment) in animals with the heart in situ. The changes produced were temporary and could be reversed by removal of the blotting paper saturated with potassium chloride. Reversal to a normal electrocardiographic pattern was hastened by washing the area affected with physiologic saline. It is of interest that Boyd and Scherf\textsuperscript{90} were able to produce similar transitory disturbances in the electrophysiologic behavior of the heart with minimal mechanical stimulations of the surface of the heart. Kisch and associates\textsuperscript{84} also reported similar disturbances following local applications of hot and cold objects. Hoff and Nahum\textsuperscript{86} also described transitory alterations in the T wave following heating and cooling of the epicardium and endocardium with small metal thermodes. The work of Winkler, Hoff and Smith\textsuperscript{91,92} should be mentioned. These authors described electrocardiographic changes following the intravenous administration of calcium chloride and potassium chloride.

The technic to be described has merit as a method for the study of certain problems related to cardiac function. The apparatus and method will be described. Some results, obtained during development and early use of the technic, will be discussed. Its advantages and disadvantages will also be reviewed.

**Apparatus**

The hypothermal instrument used (figs. 1 and 2) is a modification of an instrument described by Hass and Taylor.\textsuperscript{93} Myocardial injury is produced by creating a negative thermal gradient from the myocardium to the contiguous instrument which is cooled by expanding carbon dioxide.

The complete assembly has two functions. One function is the supply of carbon dioxide. The second function is the control of dimensions of myocardial lesions.

The supply of carbon dioxide is maintained in a standard commercial cylinder fitted with a pressure gage. The pressure in the cylinder is kept at 800–1100 pounds per square inch. A flexible metal hose, with standard fittings, connects the cylinder to the fitting at the base of the handle of the hypothermal instrument. When the instrument is assembled for use, one of the cooling plates, shown below the shaft of the instrument, is fitted to the end of the shaft. When the valve of the cylinder is opened, carbon dioxide passes through the flexible metal hose and through a small central tube in the handle and shaft of the instrument. The gas escapes through a small orifice at the

**Fig. 1.** Hypothermal instrument. The interchangeable cooling plates which have varied diameters lie on the ruler beside the shaft of the instrument. The right angle adaptor and hypothermal needle are shown below it.

**Fig. 2.** Trochar attachment for freezing instrument used in the production of interventricular septal lesions.
end of the shaft and expands opposite the inner surface of the cooling plate. Heat is abstracted from the plate and adjacent myocardium. The expanded gas escapes through eight peripheral holes at the end of the shaft and passes through the large external tube. It escapes through the orifice of the brass tube at the base of the handle.

A right angle adaptor is illustrated in the lower right hand corner of figure 1. The adaptor is used in the production of posterior lesions in the atria and ventricles. Any one of the freezing plates can be fitted to the distal end of this adaptor.

A hypothermal needle is shown in figure 1. The needle consists of a larger external needle with a closed tip and a smaller internal needle. Carbon dioxide passes through the central needle, expands and escapes in the space between the external wall of the smaller needle and internal wall of the larger needle. It can be fitted to the hypothermal instrument and is used in the production of interventricular septal lesions. A trochar having a diameter of 5 mm. and a length of 13 cm. was also used (fig. 2). It operates like the needle described above.

The three freezing plates shown on the ruler in figure 1 have diameters of 6, 15 and 20 mm. respectively. They are interchangeable and are used to vary the diameters of lesions produced.

**Method**

**General Procedure**

Dogs weighing from 8 to 15 Kg. were anesthetized with Nembutal given intravenously (30 mg. per Kg.). An M.S.A. Pneophore* resuscitator was attached to an intratracheal catheter. The intratracheal catheter was made air tight by an inflated rubber cuff surrounding the distal end of the catheter. Intrapulmonic pressure was maintained at 10 to 15 mm. Hg.

Sterile surgical procedures were rigidly followed. The heart was approached through a parasternal incision or through an intercostal space. Either pleural cavity was approached on the appropriate side of the sternum. The costal cartilages were cut medial to the internal mammary vessels. In the dog there is a prominent subpleural muscular layer in the region of the sternum. This was cut across while entering a pleural cavity. Exposure of the heart was accomplished through different incisions in the pericardium. Incisions were purposely made away from the intended site of injury of the myocardium. The visceral pericardium was anesthetized by placing circular cotton pledges saturated with 20 per cent Novocain over the site to be frozen. These pledges were left in place for three minutes.

The tip of the hypothermal instrument was placed against the moistened surface of the heart at the desired location (fig. 3A). Large coronary arteries were avoided. The valve controlling the flow of carbon dioxide was opened. Carbon dioxide flowed through the instrument continuously. When a lesion of a desired depth was produced, the carbon dioxide was turned off. The instrument was heated by spraying steam through a needle into the exhaust outlet

of the instrument. This facilitated removal of the instrument from the heart within 30 seconds. Immediately following the production of a lesion the area frozen was a firm white cylindrical mass (fig. 3B).

After the production of the myocardial damage, the pericardial sac was closed with fine silk sutures. The chest wall was closed in layers. The costal cartilages were sutured with stainless steel wire.

Postoperative pneumothorax was avoided by increasing intratracheal pressure to 30 mm. Hg as the chest was closed. The skin was closed with interrupted silk sutures. When lesions were produced in the posterior walls of the ventricles and atria, the right-angle adaptor was used. Thus, the hypothermal plate could be readily placed against the posterior surface of the heart with minimal manipulation of the heart.

Serial electrocardiographic studies were made in 40 dogs with single myocardial lesions, in 2 animals with localized pericarditis produced by suturing a piece of cellophane (20 mm. diameter) to the epicardium and in 3 animals with massive myocardial lesions produced by making three or four confluent lesions. Serial electrocardiographic studies were also made in 4 control animals who were subjected to identical surgical procedures without producing myocardial lesions. Electrocardiograms were made before, during and 24 hours after the production of interventricular septal lesions. Standard leads I, II, and III and an apical precordial lead were taken serially in 27 animals with single myocardial lesions and in the 4 control animals. In 8 animals with single lesions the three standard limb leads and four precordial leads were taken (two on each side of the chest). In 5 animals with single lesions, 2 animals with localized pericarditis (produced with cellophane) and 3 animals with massive myocardial lesions, standard limb leads, unipolar limb leads and four or more unipolar precordial leads were recorded serially.

In a few animals, silver clips were placed in the centers of lesions. The lesions, easily identified with the aid of the silver clip, were studied fluoroscopically at frequent intervals during four weeks.

Dogs were sacrificed after variable periods of time ranging from 4 to 63 days. Hearts were weighed and fixed in formalin. The lesions were studied grossly and microscopically. Volumes of lesions were determined by measuring their areas and depths. The areas were determined by measuring a template having an area equal to that of the surface of the lesions. The depths of healed, retracted lesions were estimated by measuring the thickness of normal myocardium adjacent to the lesions.

Production of Interventricular Septal Lesions

Lesions were produced in the interventricular septum by two methods. The hypothermal needle was used in the production of discrete cylindrical lesions extending through the full thickness of the interventricular septum. The needle was inserted through the wall of the right ventricle and transversely through the septum. The course of the needle was guided by palpation through the thin, compressible, right ventricular wall. After insertion of the needle into the septum, lesions were produced by allowing carbon dioxide to flow through the instrument. Hemorrhage from the needle’s puncture in the right ventricle was controlled by suturing a small piece of muscle over the wound.

Small, cylindrical, subendocardial lesions, having depths of 2 to 5 mm., were produced on the right surface of the interventricular septum by inserting a hypothermal trochar (diameter 5 mm.) into the right ventricle and placing the flat end of the instrument against a desired point on the septum (see fig. 2). Here again, the course of the instrument was guided by manual palpation through the right ventricular wall. Carbon dioxide was turned on until a lesion of a desired depth was produced. Electrocardiograms were recorded before surgery, after the chest was opened, after insertion of the freezing instrument into the right ventricle, during freezing of the septum and 10 minutes, 30 minutes, and 24 hours after septal injury.

Production of Multiple Right and Left Ventricular Lesions.

A large portion of the myocardium of either the left or right ventricle, excluding interventricular septal myocardium, was inactivated in two stages. The heart was approached through an anterolateral incision through the third or fourth intercostal space. The pericardium was opened in the usual manner. The epicardium was anesthetized by placing over the area to be frozen, for three minutes, a cotton pledget saturated with 20 per cent Novocain. Three or four transmural lesions having diameters of 20 to 25 mm. were produced at each operation. The posterior wall of the ventricle was attacked during the first operation. The anterior wall of the ventricle was damaged at the second operation. Pericardial adhesions over the lesions following the first operation presented no problem since in the case of two stage procedures the pericardial incision was made over the site to be frozen.

Quinidine (4 mg. per Kg.) given orally, one hour before surgery was used twice and seemed to be unsatisfactory for avoidance of fatalities when several myocardial lesions were produced. It successfully prevented ventricular fibrillation but animals died within 24 hours after surgery. A progressive bradycardia began during the production of lesions and terminated in cardiac arrest.

Instillation of 10 and 20 per cent Novocain into the pericardial sac was used during earlier experiments. This type of local anesthesia was later abandoned because it became apparent that not infrequently complete transmural right atrial anesthesia occurred resulting in fatal disturbances in cardiac conduction.
RESULTS

Myocardial lesions have been produced in 87 dogs. Twenty-one dogs developed fatal ventricular fibrillation or cardiac arrest shortly after lesions were produced. Nine dogs died from infection of the pleura and pericardium; 57 animals survived. Mortality decreased progressively, as technics and methods were improved. Animals survived following the production of single or multiple lesions in the right or left ventricle and the interventricular septum. Lesions were produced in the posterior wall of the right atrium with survival. Animals were sacrificed 4 to 63 days after surgery.

Single lesions, having diameters of 15 or 20 mm. and, in most instances, being transmural, were produced in 36 animals with survival. Single lesions, with survival, were made anteriorly and posteriorly in the right and left ventricle and posteriorly in the right atrium.

Lesions were made in the interventricular septum in 12 dogs. Four dogs developed immediate fatal ventricular fibrillation. Four animals had lesions in the septum demonstrable at autopsy but had no disturbances in cardiac conduction. Four animals showed disturbances in conduction. One animal showed complete atrioventricular block. Three animals showed right bundle branch block. In one instance right bundle branch block was transitory, persisting for only a few minutes.

Multiple cardiac lesions were produced in 14 dogs. Two animals developed fatal ventricular fibrillation as lesions were being produced. In two experiments, with 40 mg. of quinidine given preoperatively, animals tolerated massive destruction of the right ventricle (one animal) and of the left (one animal), without developing ventricular fibrillation. However, in these 2 animals a progressive bradycardia with final cardiac arrest occurred in 1 and 24 hours, respectively. Ten animals survived with three or four transmural lesions in the right or left ventricle (without quinidine). Volumes of myocardial damage ranged from 27 to 50 per cent of the volume of right ventricle and 30 to 40 per cent of the left ventricle (excluding the interventricular septal muscle) (fig. 4). Two animals survived with six and seven lesions in the left ventricle and 2 animals survived with seven lesions in the right ventricle. The volume of myocardial damage was about 70 per cent in each case (excluding the interventricular septal muscle). In these 4 animals three or four lesions were produced at one operation; two weeks later three additional lesions were made in each animal.

Several factors were important in the control of fatal ventricular fibrillation. Anesthetization of the epicardium covering myocardium to be frozen was an important factor. The pledget saturated with Novocaïn was left on the epicardium for three minutes before a lesion was produced. Each area to be frozen was anesthetized before a lesion was produced. Manipulation of the heart was kept at a minimum and when necessary it was done gently. The production of sudden changes in the heart’s position and filling pattern was hazardous after lesions had been produced. Nembutal given intravenously in doses greater than 30 mg. per Kg. was not desirable. Deeper anesthesia seemed to be a factor in the occurrence of ventricular fibrillation.

Fatal ventricular fibrillation was precipitated in a few instances by temporary occlusion of a major coronary artery. When proximal portions of the anterior descending branch or secondary descending branch of the left coronary artery were frozen, the flow of blood was temporarily occluded and ventricular fibrillation followed. Lesser branches were frozen frequently without fatalities. All epicardial vessels assumed normal function within a few minutes after the removal of the hypothermal instrument. Thrombosis did not occur in these vessels.

Ventricular fibrillation following the production of myocardial damage usually was irreversible and fatal. All animals with ventricular fibrillation were treated with manual cardiac massage and electric stimuli. One ampere of 110 volt current (Hooker and co-workers*) was passed transversely through the ventricles for from 0.2 to 1.0 second. Copper disks having diameters of 1 inch were placed securely against the lateral aspects of the ventricles. Frequently, electric stimulation was used five or six times in each case. Manual cardiac massage was used
between electric stimuli. One of 21 dogs survived longer than 12 hrs.; it was sacrificed 42 days after surgery. In this single instance ventricular fibrillation was corrected after having been present 20 minutes. Usually animals developed asystole within 30 or 45 minutes after the onset of ventricular fibrillation. Intracardiac injections of adrenaline, 1 cc. of 1:1000 solution, or intracardiac injections of Novocain, 2 cc. of a 10 per cent solution, were used by Hooker and his co-workers to correct electrically induced ventricular fibrillation in hearts without myocardial damage. In all of the animals mentioned above, myocardial damage was present. In two experiments ventricular fibrillation occurred before myocardial damage was produced. This was corrected by electric shock.

The problem of postoperative pneumothorax was not encountered. Postoperative hydrothorax only occurred when infections oc-

in some instances with no apparent benefit. Hooker and his associates describe excellent results in correction of ventricular fibrillation by injecting 5 cc. to 10 cc. per pound of a solution containing calcium chloride, 0.046 per cent, in 0.9 per cent saline containing heparin and warmed to body temperature. They added 2 cc. of 1:1000 adrenaline to this mixture and injected it centrally into the carotid artery under a pressure of 150 mm. Hg. We did not use this method. It should be pointed out that occurred in the pleura. Pneumothorax was carefully avoided by increasing intratracheal pressure to 30 mm. Hg. as the chest wound was being closed. The wound was tightly closed simultaneously with an expiratory excursion of the chest.

Electrocardiographic Studies

Electrocardiographic studies were useful for the study of conduction disturbances during and after the production of interventricular

Fig. 4. Anterior view (A) and right lateral view (B) of a dog's heart showing multiple healed lesions in the right ventricle. Fifty-five per cent of the extraseptal myocardium of the right ventricle is replaced by a thin fibrous scar which in A shows some aneurysmal dilatation. In B the margin separating the thin scarred wall from normal myocardium can be seen.
septal lesions. However, they were of practically no value for evaluation of age and volume of myocardial necrosis in the right or left ventricle. Lesions produced by this method invariably were accompanied by local areas of pericarditis which alone change the electrocardiogram. The two processes, myocardial necrosis and localized pericarditis, resulted in a mixed electrocardiographic picture. Only after subsidence of pericarditis (10 to 14 days) was localization at all feasible. Posterior and anterior myocardial lesions could usually be differentiated then. Finer localization was impossible.

Lesions were made in the posterior wall of the right atrium in 5 dogs. Two immediately developed fatal ventricular fibrillation. Two of 3 animals showed an inversion of the P wave through the eleventh postoperative day. The P wave had returned to normal (in one animal) on the twenty-ninth postoperative day.

**Fluoroscopic Studies**

Fluoroscopic studies showed paradoxical behavior of transmural myocardial lesions. During systole lesions showed mild aneurysmal dilatation. During diastole, since lesions were nonviable, there was no dilatation of lesions. Systolic aneurysmal dilatation was more marked during the first 14 days after myocardial injury. After fibrosis of lesions had occurred, dilatation during systole was less marked. These findings were more striking in animals with right ventricular lesions.

**Pathologic Studies**

Postmortem examination of animals, after myocardial damage had been produced, showed adherence of the pericardial sac to the line of closure of the healed thoracic surgical wound. Pleural adhesions were surprisingly minimal and consisted of a few fibrous bands between the anterior margin of the lung and the healed thoracic wound or the pericardium. Adhesions along the line of closure of the pericardial sac to the epicardium and from the surface of the lesion to the pericardial sac were a constant finding.

Immediately following the production of a lesion the area frozen was a firm white cylindric mass (fig. 3B). As the lesion thawed, vasodilatation became conspicuous in the epicardium and the myocardium. Widespread pericapillary hemorrhage and edema were present, but sharply limited by the boundaries of the lesion. Spontaneous hemostasis occurred promptly.

Cardiac lesions, a few hours old, showed a slight increase of edema and a mild fibrinous exudate on the epicardium overlying the inactivated myocardium. Microscopic examination showed a few agglutination thrombi in small blood vessels. The cells of cardiac muscle showed numerous minor structural alterations similar to those found in acute human myocardial infarcts. There were few infiltrating leukocytes. Fibrocytes, endothelial cells and collagen were surprisingly well preserved. No thrombi were found adjacent to the endocardium even when this structure was involved by the lesion.

The study of the evolution of the lesions showed progressive resorption of extravasated blood and degenerated cardiac muscle fibers. From the fourth through the fourteenth day calcium salts were present in degenerating cardiac muscle cells at the margins of lesions. These were apparently resorbed and were not seen in lesions older than 14 days (figs. 5 and 6). Fibroblasts and newly formed capillaries became conspicuous (fig. 6). Collagen was gradually deposited. There was never any suppuration, rupture of the myocardial wall or significant anatomic aneurysmal dilatation. A thin, contracted, depressed cylindric scar was the final result of healing (fig. 7). The principal difference at any stage between these lesions and human myocardial infarcts due to occlusion of coronary arteries was that the experimental lesions were always homogeneous and very sharply defined.

**Discussion**

The methods described have certain advantages over other methods for the production of myocardial injury. Complete necrosis of muscle fibers in a given area can be produced with certainty. Transmural lesions in any cardiac chamber can be made without danger of cardiac rupture, significant aneurysmal dilatation or intracardiac thrombosis. Myocardial necrosis can be selectively produced as to volume and location. Volumes of myocardial damage
Fig. 5. A transmural anterior left ventricular lesion in a dog's heart. Calcium salts can be seen at the margins of the lesion which is one week old. The coronary vessel shown passing over the surface of the lesion was not thrombosed. It was frozen when the lesion was made. (The use of color in this illustration has been made possible by a grant from Wyeth Incorporated to the publication fund of the American Heart Association.)

Fig. 6. Photomierograph of margin of lesion shown in fig. 5. The masses of dark granular material separating the lesion from normal myocardium are calcium salts stained with hematoxylin. In the lesion there is complete necrosis of muscle cells. Fibroblasts and newly formed capillaries are conspicuous.

can be predicted quite accurately and can be measured accurately at postmortem examination. Large portions of the right or left ventricle can be inactivated by successive operations in chronic animal preparations. These animal preparations with massive destruction of a ven-
tricle are now being used in the study of chronic cardiac failure.

Discrete, small interventricular septal lesions, which are readily detectable at post-mortem examination, were produced on the right side of the septum and could be produced on the left side with this method. In some cases disturbances in cardiac conduction were observed. Lesions produced by this method, with electrocardiographic studies, offer an excellent pathologic-physiologic method for the study of the cardiac conduction mechanism. The sinoauricular node could be approached through the posterior wall of the right atrium by this method.

The method has disadvantages. As the results indicate, the mortality rate in these experiments was high. However, most of the unsuccessful experiments occurred during the first half of the study. As techniques improved the mortality rate decreased markedly. Local pericarditis at the site of myocardial lesions and the site of closure of the pericardial sac occurred regularly. The superimposition of electrocardiographic changes due to localized pericarditis upon those due to myocardial necrosis makes electrocardiographic studies difficult to interpret.

**Summary**

A method for the production of controlled myocardial injury in dogs has been described. Permanent inactivation of large or small quantities of myocardium was produced with an instrument cooled to −60°C by expanding carbon dioxide. Transmural lesions were produced in any cardiac chamber, including the right atrium, without danger of rupture, aneurysmal dilatation or intracardiac thrombosis. Quantities of myocardial damage were predicted quite accurately and measured accurately post mortem. Necrosis of muscle cells was complete throughout the lesions produced. Large portions of the left or right ventricle were inactivated giving a chronic animal preparation suitable for study of cardiac failure.

Lesions produced by this method are unsatisfactory for refined electrocardiographic analysis of myocardial necrosis, because of interference incidental to pericarditis.

A method for the production of small discrete interventricular septal lesions with a cold needle has been described. The size and location of lesions was controlled. Disturbances in cardiac conduction were produced by this method. Lesions produced by this method were easily defined, grossly and microscopically.

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