Maintenance of the Heart Beat by Perfusion of the Coronary Circulation with Gaseous Oxygen *

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The ability of mammalian tissues and organs to utilize oxygen when perfused through the vascular system in the gaseous state is a basic physiologic problem of considerable importance. Despite this fact only a minimal amount of data is available concerning the subject. Several years ago Bunzl [2] demonstrated that the spinal cord of the decerebrate frog could be maintained in a reflexly active state for as long as 24 hours when the vascular system was perfused with a mixture of gaseous oxygen and carbon dioxide. More recently Burns, Robson, and Smith [3] have shown that the isolated rabbit heart perfused with oxygen maintained a beat for more than three hours. The data reported in the present study concern a group of experiments designed to evaluate the effect of perfusion of the dog heart with gaseous oxygen. Observations have been made both on the isolated heart and the heart remaining intact within the chest.

Methods and Results

In the first group of experiments 24 dogs weighing 7 to 10 Kg. were used. The animals were lightly anesthetized with sodium pentothal and respiration was maintained through an endotracheal tube attached to a respirator supplied with oxygen. The chest was entered through a bilateral thoracotomy in the fourth interspaces with division of the sternum. The right and left coronary arteries were isolated at their origin from the aorta. A strain gauge arch was then sutured to the ventricular surface for direct measurement of contractile force.[1] An electrocardiographic electrode was placed in the interventricular septum near the apex. The heart was then quickly removed from the chest together with the proximal portions of the great vessels. Gregg coronary cannulae were introduced into the aorta and passed into the right and left coronary orifices and secured with a ligature. This procedure was performed within two minutes during which time weak cardiac contraction continued. The heart was then transferred to a transparent Plexi-glass unit kept at 37° C. with electric heating tape. High humidity was maintained by a constant saline vaporizer (Fig. 1). A continuously recording telethermometer was placed into the ventricular cavity and the electrocardiographic and strain arch leads were attached to an oscilloscope and a recorder. Blood was collected from a donor and placed in a constant-temperature water bath and perfused into the coronary cannulae at a mean pressure of 100–150 mm. Hg. This was continued for several minutes

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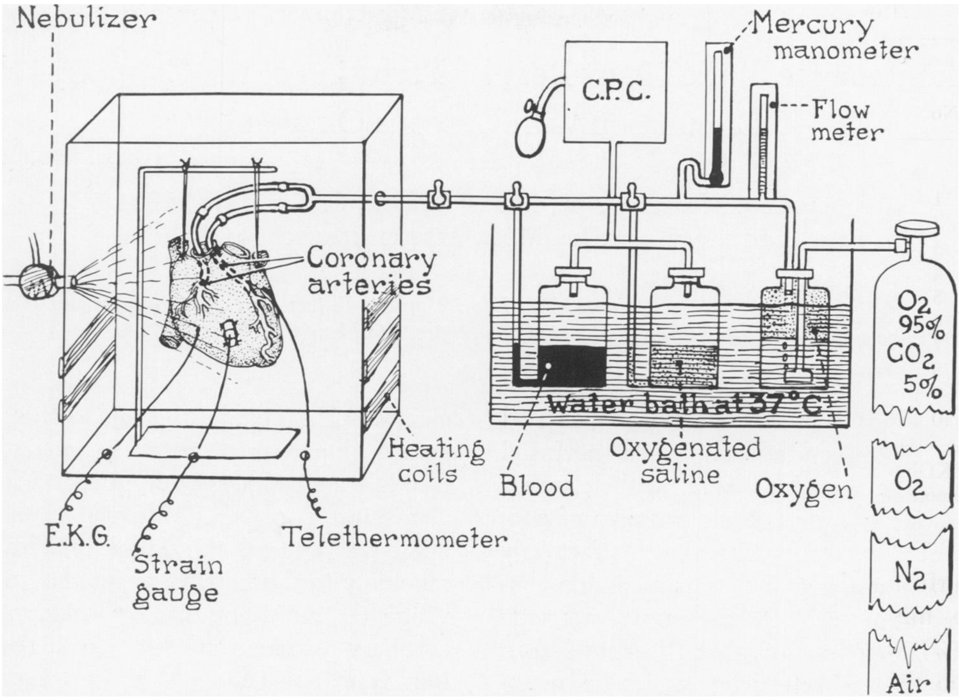

FIG. 1. Diagrammatic illustration of method employed for perfusion of the coronary circulation with gaseous oxygen. (See text for description of details.)

to permit the heart to recover from the short period of anoxia which resulted from the cannulation and transfer to the plastic chamber. After the onset of blood perfusion, cardiac contraction characteristically returned to normal strength. After restoration of a strong beat the perfusion was then changed from blood to a solution of oxygenated saline. This removed the blood from the coronary circulation, and when the venous return from the coronary sinus became clear, the perfusion was changed to humidified oxygen (95%) and carbon dioxide (5%) supplied by a gas cylinder. A diagrammatic illustration of the experiment as performed is shown in Figure 1. Oxygen was perfused at a pressure of 80 to 120 mm. Hg with a flow of 300–1,000 cc. per minute. The heart action was constantly monitored by direct observation through the transparent Plexi-glass chamber and by the electrocardiographic and strain gauge arch records on an oscilloscope. Recordings of these tracings were made at frequent intervals.

Among the ten animals in which the isolated heart was perfused with moist oxygen (95%) and carbon dioxide (5%), the heart continued to beat for periods varying from two and one-half to eight hours. The average duration of a visible heart beat in the ten hearts perfused with gas alone was five hours and seven minutes (Table 1). Cardiac contraction characteristically remained quite forceful for the first two to three hours and then became gradually weaker. The rate usually ranged from 60 to 80 during the early period and became slower with the passage of time. An electrocardiogram illustrating the typical pattern and rate change over a period of time is shown in Figure 2.

In a group of control studies identical experiments were performed up to the point

TABLE 1. *Perfusion of Isolated Heart with Gaseous Oxygen (95%) and Carbon Dioxide (5%)*

No.	Heart Weight (Gm.)	Duration of Visible Heart Beat		Duration of Electrocardiographic Activity		
		Hours	Minutes		Hours	Minutes
1	100	2	39	*		
2	78	4	54	*		
3	59	4	32	*		
4	35	6	7	Vent. fibrillation	6	7
5	60	3	48	Vent. fibrillation	3	48
6	60	11	30**	Vent. fibrillation	11	30
7	80	4	1	Vent. fibrillation	4	1
8	55	8		Asystole	12	12
9	47	7	50	Asystole	9	12
10	73	4	30	Vent. fibrillation	4	30

* EKG not continued in these hearts after cessation of visible cardiac contraction.
** Blood was perfused in No. 6 for 3 minutes after 3 hours on oxygen-carbon dioxide perfusion.

of perfusion with the oxygen-carbon dioxide mixture. In three hearts nitrogen (100%) was used instead of the oxygen-carbon dioxide mixture and was perfused until cardiac arrest occurred. The heart beat rapidly became weak and completely ceased in four, 15, and 28 minutes respectively (Table 2). In one heart which was perfused with the oxygen-carbon dioxide mixture for 30 minutes with maintenance of a forceful beat, the perfusion was switched to nitrogen (100%) and ventricular fibrillation occurred in 12 minutes. Perfusion with blood and then gaseous oxygen was followed by the return of strong ventricular contraction. In another heart oxy-

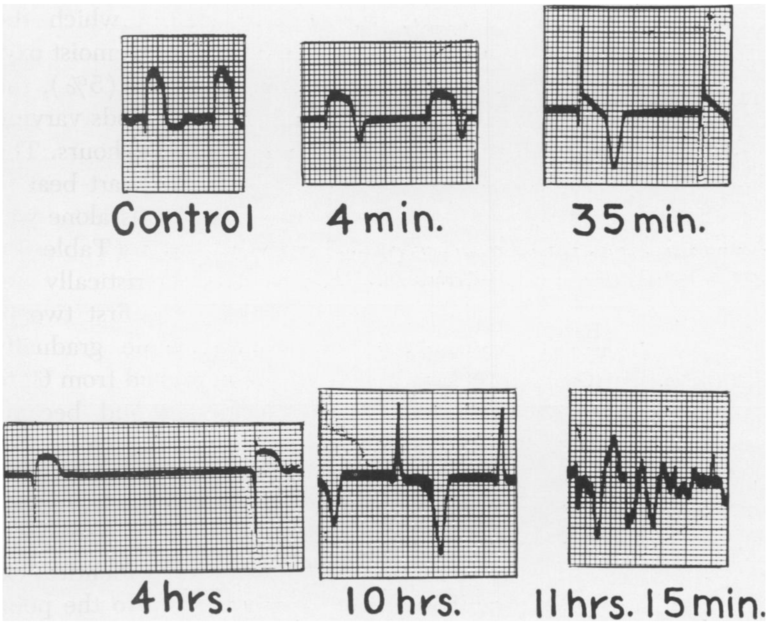

FIG. 2. Electrocardiograms recorded with an exploring electrode inserted into the interventricular septum with the remote electrode on the tip of th left atrial appendage. Paper speed 25 mm./sec. and amplitude 1 mv./mm. An essentially regular rhythm is maintained during perfusion of oxygen and carbon dioxide although the rate varies and occasional premature beats occur after the first hour. The rapidity and short duration of the QRS deflections suggest that the pacemaker is supraventricular and that intraventricular conducation is little if any delayed. Ventricular fibrillation appeared after 11 hours and 15 minutes of gaseous perfusion.

TABLE 2. *Control Studies on Gaseous Perfusion of the Isolated Heart*

Gas Perfused	No.	Heart Weight (Gm.)	Duration of Beat (minutes)	EKG (minutes)
Nitrogen	1	54	4	
(100%)	2	53	28	30 (asystole)
	3	92	15	82 (asystole)
No perfusion	1	61	8	—
of gas after	2	40	8½	—
blood and	3	52	20	20 (asystole)
saline	4	71	28	33 (asystole)
	5	95	27	32 (asystole)

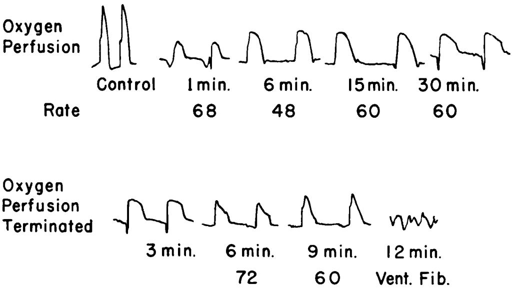

FIG. 3. Electrocardiogram showing regular P-QRS-T complexes during perfusion of oxygen. After 30 minutes the perfusion was discontinued and ventricular fibrillation developed 12 minutes later.

gen-carbon dioxide was perfused for 30 minutes at which time the oxygen perfusion was terminated. Ventricular fibrillation occurred 12 minutes later (Fig. 3).

In five other hearts nothing was perfused through the coronary circulation after the blood and saline, and the heart was observed until arrest occurred. Cardiac contraction ceased in these hearts in 8, 8½, 20, 27, and 28 minutes respectively (Table 2). Oxygen (100%) was perfused in two hearts with maintenance of the beat for three hours and four hours and ten minutes. Compressed air was perfused in three hearts with maintenance of the beat for four hours and 15 minutes, four hours and 30 minutes, and five hours and 50 minutes respectively. In one animal the moisture was removed from the oxygen-carbon dioxide perfusion mixture by passage through anhydrous calcium sulfate and the beat continued for three hours and 15 minutes. The data obtained in the above control studies are shown in Table 3.

In the second group of experiments the effect of oxygen perfusion of the coronary circulation with the heart remaining *in situ* within the chest was studied in 12 animals. Pentothal was used as the anesthetic since it has been demonstrated that barbiturates do not appreciably influence adrenergic amines.[7] Respiration was maintained through an endotracheal tube attached to a respirator supplied with oxygen. The chest was entered through a bilateral thoracotomy in the fourth interspace with transsection of the sternum. A brass cannula was inserted into the left subclavian

TABLE 3. *The Effect of Perfusion of the Isolated Heart with Oxygen (100%), Compressed Air, and Anhydrous Oxygen (95%)-Carbon Dioxide (5%)*

Gas Perfused	No.	Heart Weight (Gm.)	Duration of Beat	Duration of Electrocardiogram
Oxygen (100%)	1	73	3 hours	3 hrs. (vent. fib.)
	2	66	4 hrs., 10 min.	4 hrs., 10 min. (asystole)
Compressed air	1	68	5 hrs., 50 min.	6 hrs., 50 min. (asystole)
	2	86	4 hrs., 15 min.	4 hrs., 15 min. (asystole)
	3	57	4 hrs., 30 min.	5 hrs., 30 min. (asystole)
Anhydrous oxygen (95%) Carbon dioxide (5%)	1	74	3 hrs., 15 min.	3 hrs., 15 min. (vent. fib.)

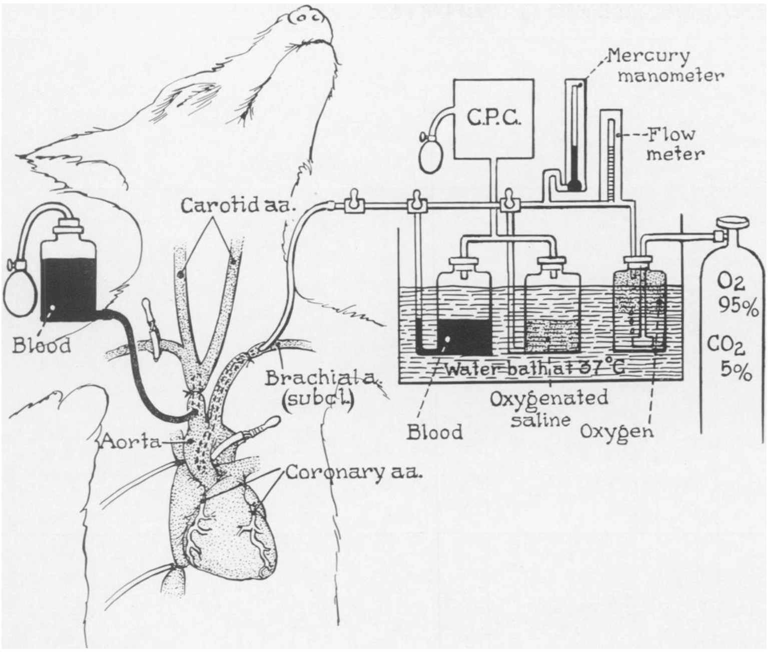

FIG. 4. Diagrammatic illustration of method employed for perfusion of the intact heart with gaseous oxygen. (See text for description of detail.)

artery and passed into the proximal aorta and tied in place with the assumption that the aortic valves would remain competent and prevent the perfused gas from entering the left ventricle. Heparin (100 mg.) was administered intravenously. A catheter was placed in the femoral artery for continuous recording of arterial pressure. The electrocardiogram was recorded from limb leads. The aortic cannula was attached to a reservoir system for perfusion of blood, saline, and the oxygen-carbon dioxide mixture. A diagrammatic illustration of the procedure as performed is shown in Figure 4. The brain was separately supplied with oxygenated donor blood and perfused from a gravity bottle. The venous blood returned through cannulae in the external jugular veins. An encircling ligature was placed around the pulmonary veins, and the vena cavae, pulmonary artery, and aorta were occluded. The right and left atrial appendages were cannulated and permitted to remain open for free drainage. After the heart became empty, blood was perfused into the coronary circulation from the reservoir until a vigorous beat was restored. When the saline from the coronary sinus became clear, the perfusion mixture was changed to gaseous oxygen (95%) and carbon dioxide (5%). The gas perfusion was continued for 25–30 minutes, during which time the systemic venous return was occluded and the cerebral circulation was perfused from a reservoir of donor blood. At the end of this period the gas in the coronary circulation was removed by perfusion of saline and followed by blood at a pressure of 100–120 mm. Hg. The tapes occluding the vena cavae were removed and the venous return was permitted to enter the heart. The carotid perfusion from the reservoir was discontinued and the carotid cannulae were removed. The femoral arterial pressure was continuously recorded during the recovery period. An attempt was made to restore the normal circulation in each of the animals in this group and was accomplished in nine of the twelve with maintenance of a heart beat for one to 48 hours. The mean arterial pressure after restoration of ventricular contraction ranged from 40

TABLE 4. *Perfusion of the Heart in Situ Within the Chest With Gaseous Oxygen and Carbon Dioxide*

No.	Weight (Kg.)	Control Mean Arterial Pressure (mm. Hg)	Total Perfusion Time (Mins.)	Postperf. Mean Arterial Pressure (mm. Hg)	Duration of Heart Beat
8	10.0	100	25	100	2½ hrs.
9	9.5	90	28	90	2½ hrs.
10	15.6	105	27	60	½ hr.
11	17.3	86	25	100	5 mins.
12	10.5	90	26	70	4 hrs.
13	8.6	—	25	—	Immediate vent. fib.
14	7.2	—	25	100	48 hrs.
15	8.1	100	25	40	5 mins.
16	8.6	140	25	105	1 hr.
17	8.1	90	25	65	1½ hrs.
18	7.4	115	25	90	3 hrs.
19	7.1	140	25	120	5 hrs.

to 120 mm. Hg with an average of 84 mm. Hg (Table 4). In one animal ventricular fibrillation occurred immediately following reversion to blood perfusion and in two others the heart beat was restored, but ventricular fibrillation ensued shortly thereafter. An illustration of the course following gaseous perfusion of the heart in situ is shown in Figure 5. The survival figures in this group of animals are not considered to represent accurately the true situation inasmuch as the period of anoxia for the remainder of the body was prolonged and the method of cerebral perfusion not optimal. The majority of the animals did not regain consciousness and cerebral anoxia and possibly air embolism were suspected. Further work on this portion of the study is in progress.

Discussion

Under normal physiologic conditions oxygen is supplied to the tissues by the oxygen contained in hemoglobin and that which is in physical solution in the blood. Under experimental conditions oxygen may be provided by electrolyte solutions highly saturated with oxygen and perfused into the vascular system. Oxygen utilization of thin slices of tissue may be studied *in vitro* by the Warburg technique in which the sections are constantly bathed in an oxygen-rich solution. In each instance oxygen is transported in a liquid medium. Until recently little was known concerning the ability of mammalian tissues to utilize oxygen in the *gaseous* state. In 1954 Bunzl and associates [2] first found that the spinal cord of the decerebrate frog could be maintained in a reflexly active state for 24 hours when the vascular system was perfused with gaseous oxygen and carbon dioxide. These observers had previously noted that perfusion with oxygenated saline solutions led to the rapid formation of edema and had sought a method to prevent it. The perfusion of gaseous oxygen allowed maintenance of normal reflex activity of the spinal cord without edema and permitted the isolated administration and evaluation of drugs in small volumes.

Further studies employing perfusion of gaseous oxygen were done by Burns, Robson, and Smith and published in 1958.[3] They perfused the isolated hind limb of a cat through the femoral artery with a mixture of moist, warm, oxygen and carbon dioxide. With this technique the tibialis mus-

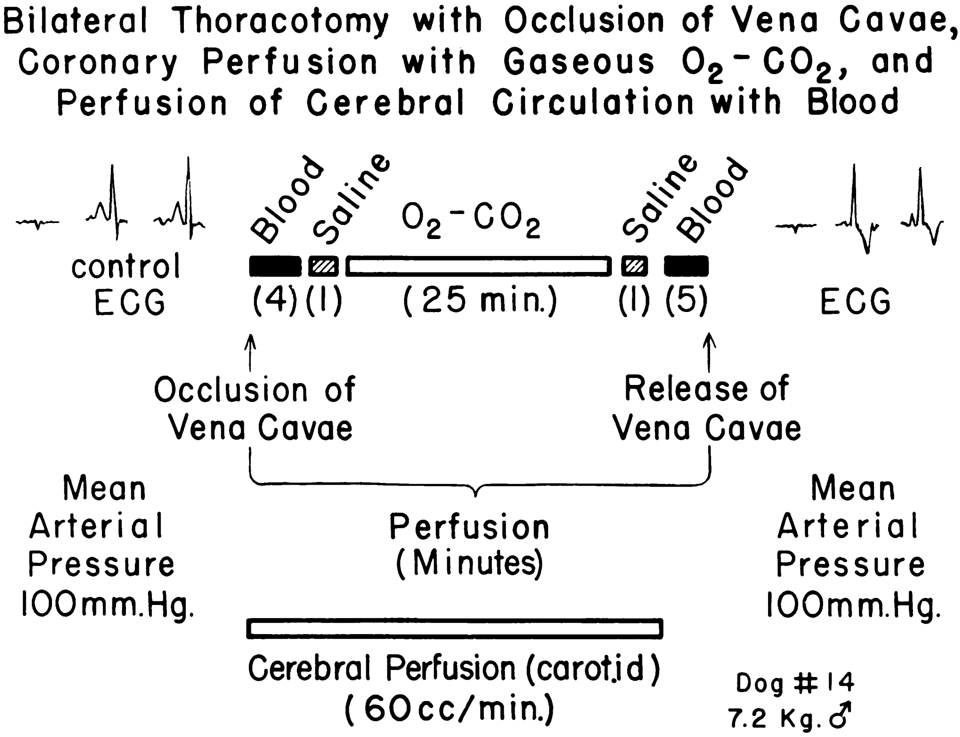

FIG. 5. Diagrammatic illustration of gaseous perfusion of the coronary circulation with oxygen-carbon dioxide in the intact heart. Gas was perfused for 25 minutes with interruption of the circulation and separate perfusion of the brain with blood. Cardiac function returned with a sinus rhythm and a mean arterial pressure of 100 mm. Hg. The heart continued to beat for 150 minutes.

cle was excited at ten second intervals by supramaximal, single stimuli of the sciatic nerve with maintenance of an undiminished twitch tension for three to four hours. Their studies also showed that the isolated rabbit heart continued to beat for periods of more than three hours when the coronary circulation was perfused with gaseous oxygen.

The marked dependence of normal performance of the mammalian heart upon a continuous and adequate supply of oxygen is well established. In the experimental laboratory total deprivation of oxygen to the intact dog heart, produced by acute ligation of both the right and left coronary arteries, results in ventricular fibrillation or asystole within several minutes, and this state is irreversible in the absence of resuscitative measures. Gregg [4] has shown that in the normal beating dog heart the oxygen consumption is 8 to 10 cc. per 100 grams of left ventricle per minute. This high rate of oxygen utilization of the myocardium is illustrated by a consideration of the fact that although the heart represents approximately 0.5 per cent of the total body weight the heart utilizes approximately 10 per cent of the total oxygen consumption.

McKeever, Gregg, and Canney [6] have shown further that the average oxygen usage of the empty but beating heart is 3.4 cc. per 100 grams of left ventricle per minute or approximately 38 per cent of the requirement for the normal beating heart. They also showed that the perfused, nonbeating heart (in a state of asystole) utilized 2.0 cc. per 100 grams of left ventricle per minute. These studies indicate that the basic oxygen requirements of the myocardium even during asystole are appreciable and that the empty but beating heart utilizes more than a third the amount of oxygen necessary for the normal beating heart.

Recent studies of Kardesch, Hogancamp, and Bing [5] indicate further the sensitivity of the heart to anoxia. They studied the effects of complete ischemia on the spontaneous ventricular membrane action potential of the whole dog and rabbit heart. A completely ischemic state was produced by cessation of coronary circulation and the continuous bubbling of nitrogen. They found that spontaneous membrane action potentials persisted for 20 minutes following cessation of coronary perfusion. From metabolic studies Winterscheid, Vetto, and Merendino [8] conclude that in the arrested, unperfused heart aerobic metabolism is probably completed after two minutes and that anaerobic metabolism contributes an almost negligible amount. These and other studies clearly demonstrate the dependence of the heart upon oxygen and make it an organ which is quite suitable for an evaluation of the utilization of oxygen perfused in the gaseous state.

In the present study the coronary circulation of the isolated dog heart was perfused with a mixture of oxygen and carbon dioxide for prolonged periods with continuous observation and recording of the ventricular contraction pattern and the electrocardiogram. In ten hearts studied by this method the heart beat was maintained for two and one-half to eight hours with an average of five hours and 45 minutes. In several instances electrocardiographic activity characteristic of ventricular conduction persisted for an additional period after the cessation of the visible heart beat. In the majority of the hearts the beat remained strong and regular for the first two to four hours. After this time the beat became noticeably weaker and usually terminated in ventricular fibrillation. In two instances the visible heart beat ceased at eight hours with persistence of electrocardiographic activity for nine and 12 hours and both ended in asystole. Further evidence that the gaseous oxygen is utilized by the heart is shown by the contrast occurring following the perfusion of 100% nitrogen which produced arrest in an average of 16 minutes. When no gas was perfused, arrest also occurred in an average of 16 minutes. The effect of interruption of oxygen perfusion was also demonstrated with ventricular fibrillation occurring in 12 minutes.

The second group of experiments was designed to evaluate the possibility of reestablishment of the normal coronary circulation after perfusion of the heart for a period with gaseous oxygen. In the past much concern has been directed toward gaseous embolism of the coronary circulation. The data show that it is possible to change the perfusion of the coronary circulation from blood to gas and to later permit the heart to resume normal circulation. Good ventricular contraction with maintenance of a satisfactory mean aterial pressure was noted in the majority of animals. The studies are not considered to accurately reflect the true results of long term survival due to the prolonged anoxia of other organs which occurred during the perfusion.

Summary

Experimental studies have shown that the beat of the isolated dog heart may be maintained for periods up to eight hours by perfusion of the coronary circulation with oxygen in the gaseous state. In some instances electrocardiographic activity continued after cessation of visible ventricular contraction and was observed for periods up to four hours after the last visible beat. Perfusion of the heart *in situ* within the chest with oxygen has been performed with later reversion to blood and re-establishment of the normal circulation. The studies indicate that oxygen perfused in the gase-

ous state is utilized by the heart and that cardiac contraction may be maintatined for prolonged periods when the heart is perfused with oxygen alone.

Acknowledgment

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Bibliography

1. Boniface, K. J., O. J. Brodie and R. P. Walton: Resistance Strain Gauge Arches for Direct Measurement of Heart Contractile Force in Animals. Proc. Soc. Exper. Biol. and Med., **84**:263, 1953.
2. Bunzl, A., A. S. V. Burgen, B. D. Burns, N. Pedley and K. G. Terroux: Methods for Studying the Reflex Activity of the Frog's Spinal Cord. Brit. J. Pharmacol., **9**:229, 1954.
3. Burns, B. D., J. G. Robson and G. K. Smith: The Survival of Mammalian Tissues Perfused with Intravascular Gas Mixtures of Oxygen and Carbon Dioxide. Can. J. Biochem. Physiol., **36**:499, 1958.
4. Gregg, D. E.: Regulation of the Collateral and Coronary Circulation of the Heart. In Proceedings of the Harvey Tercentary Congress, London, Blackwell Scientific Publications, Ltd., 1958.
5. Kardesch, M., C. E. Hogancamp and R. J. Bing: The Effect of Complete Ischemia on the Intracellular Electrical Activity of the Whole Mammalian Heart. Circulation Research, **6**:715, 1958.
6. McKeever, W. P., D. E. Gregg and P. C. Canney: Oxygen Uptake of the Nonworking Left Ventricle. Circulation Research, **6**:612, 1958.
7. Richardson, J. A., E. F. Woods and A. K. Richardson: Plasma Concentrations of Epinephrine and Non-epinephrine During Anesthesia. J. Pharm. and Exp. Therap., **119**:378, 1957.
8. Winterscheid, L. C., R. R. Vetto and K. A. Merendino: Myocardial Carbohydrate Metabolism During Induced Cardiac Arrest and Post-Arrest Perfusion. Ann. Surg., **148**:481. 1958.

Discussion

Dr. Frank Gerbode: I believe these experiments of Drs. Sabiston, Blalock and co-workers are not only extremely interesting, but may present some ultimately valuable leads toward the maintenance of metabolism in the isolated heart, particularly during open heart surgery.

There are a number of questions which arise after reading of their beautifully conceived experiments. I am sure, incidentally, that you will all appreciate fully the value of a nicely designed experiment, as this is, and I wish to compliment them on this as well.

Questions which arise, of course, other than the fundamental one which has been shown to be that oxygen will maintain the metabolism of the heart in this way are, for example: what the metabolism of the heart might be when stopped with potassium for a certain period of time; what is the effect on the cold heart under these circumstances; what metabolic waste products result from lack of nutritional support?

I should also like to know what the pathologic examination of the myocardium and of the intima might show after these experiments, although, of course, from a practical point of view the experiments need not have been of this duration.

I believe that we have all come to the conclusion in open heart surgery that although we can isolate the heart, stop it, and perhaps keep it in a reasonably satisfactory metabolic state for half an hour with potassium or anoxic arrest, beyond this the heart does not really recover quite as well. For this reason other metabolic adjuncts have to be added, and perhaps this is one.

Again, I would like to compliment the authors on the presentation of the material. I believe the addition of the movie strip showing the beautifully isolated heart certainly has added enormously to the value of the presentation.

Dr. K. Alvin Merendino: Mr. Chairman, Members and Guests: There is very little that one can add to this beautiful presentation by Dr. Sabiston. It obviously represents an area which needs further investigation.

From our studies of the isolated heart with regard to glucose metabolism, one must anticipate here a tremendous glycogen debt within the cells. It is conceivable that other substrates may be utilized for metabolism if glycogen depots are depleted. The crux of the matter is whether hearts perfused with humidified oxygen can at some later period be resuscitated. Conceivably, there may be alterations in intracellular pH which may be un-