Chemical Changes in Blood and Heart Tissue in Experimental Coronary Occlusion.

Albert Jerome Bocage
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A Dissertation

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Doctor of Philosophy

in

The Department of Physiology

by

Albert Jerome Bocage
B.S., Xavier University of New Orleans, 1957
M.S., Louisiana State University, 1962
August, 1964
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ABSTRACT

This study consisted of investigations into the chemical alterations in the plasma of the dog during the first several hours following coronary occlusion, and their relationships to the alterations in the composition of the heart tissue. In conjunction with these investigations, measurements have been made of retrograde blood composition, oxygen saturation, flow and pressure.

The results demonstrate increases in the plasma concentrations of the following substances after coronary occlusion: lactate, total amino acids, alanine, glucose, lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GO-T), calcium, and pyruvate. These changes are accompanied by a decrease in bicarbonate, a decrease followed by an increase in magnesium, and no change in chloride. The similarity between the effects of coronary occlusion and those of exogenous catecholamines upon the composition of the plasma strongly suggest that the changes in plasma composition following coronary occlusion are mediated by the catecholamines. The effect of dibenzyline (phenoxybenzamine HCl) pre-treatment upon the plasma composition following coronary occlusion supports this postulation. The effect of bilateral adrenalectomy upon the changes in plasma composition following coronary occlusion indicate that the catecholamines are probably liberated from post-ganglionic nerve endings. Differences between retrograde and arterial plasma concentrations have been noted and their relationship to myocardial metabolism discussed.
Measurements of the oxygen saturation of the retrograde blood yielded values which tended to be slightly lower than those of the arterial blood obtained at the same time. Wide variations in the retrograde blood flow and peripheral coronary pressure were observed.
INTRODUCTION

'The heart as a pump' is a phrase often used to head chapters and sections of physiology texts. It is used because it succinctly describes the primary function of the heart, the pumping of blood. The ultimate objective of extensive investigation has been the determination of how and why accidents and disease states adversely affect this function. A great deal of this investigation has been devoted to the study of the effects of coronary occlusion. The result of such an occlusion is the cessation of blood flow to a portion of the heart muscle. A study of certain of the more immediate effects of this condition of relative ischemia constitutes the main objective of this work.

The three basic activities of the heart, i.e. electrical, mechanical, and chemical, constitute the parameters disrupted by the ischemia resulting from coronary occlusion, and are the subjects under study following experimental coronary obstruction. The three activities are, of course, intricately interrelated and disruption of one of the systems necessitates disruption of the others. Perhaps the most important parameter concerned in the study of coronary occlusion could be said to be chemical because the deprivation of the most vital metabolic constituent (oxygen) is the basic underlying factor leading to the disruption of the integrity of myocardial activity. The study of these parameters is not, of course, as simple as this very broad scheme might indicate. The intricacy of the intercorrelation of the parameters, together with the complexities inherent in each of the systems involved, leads to a study
of the basic phenomena underlying metabolic activity of all biological cellular systems.

Because of the volume of work that has been done on the heart and for the sake of brevity, no attempt will be made to provide a complete history of coronary occlusion and all that it entails. It is hoped that the compilation of information directly, or indirectly, pertinent to this work in the form of a background sketch will suffice to introduce the subject at hand. An attempt has been made to present this information in some semblance of order, but, because the subjects of some of these studies overlap into different sections this has not always been possible.

I. Alterations of Plasma and Tissue Constituents Following Coronary Occlusion

It is said that the eyes are the windows of the soul; so too, the blood can be regarded as a 'window' through which alterations in the body's activity may be observed. The utilization of this window by those interested in the aftermath of coronary occlusion has been extensive. Alterations in various of the plasma components have been studied in the search for methods to increase the reliability of diagnosis and prognosis in clinical cases of coronary obstruction and to search for the scientific truth underlying the physiological and biochemical phenomena associated with this condition. Changes in the composition of heart tissue have been investigated in order to determine tissue components that are altered, rates and magnitudes of their alterations and the relationships of tissue changes to plasma changes following coronary occlusion.

A. Electrolytes

Alterations in concentrations of electrolytes following coronary occlusion have been extensively studied in both heart muscle and plasma.
The depletion of potassium from the muscle has been demonstrated by alterations in the content of the muscle (Russell et al., 1961, a) and of the blood plasma emanating from local veins and the coronary sinus following coronary occlusion (Harris et al., 1954 and Cherbakoff et al., 1957). The accumulation of sodium in the infarcted muscle following coronary occlusion has been demonstrated by analysis of alterations in its composition by Russell et al. (1961, a). Histochemical analysis has shown the accumulation of calcium ions in the area comprising the border zone between normal and infarcted tissue (Wartman, 1963). Cummings (1960) has measured the depletion of magnesium from the tissues and its appearance in the coronary sinus and arterial blood plasma 8 - 11 hours after coronary occlusion.

The abilities of these ions to produce changes in the excitability of the cardiac muscle fibers are well known. The excitant properties of potassium and the depressant properties of magnesium on ectopic activity following coronary occlusion have been illustrated by Harris et al. (1954), Harris et al. (1953) and Carden and Steinhaus (1957).

B. Enzymes

The pioneering work of La Due and Wroblewski (1954, 1955) opened up a new field in the diagnosis of myocardial infarction. The relationship between the maximum increase in the activity of glutamic oxaloacetic transaminase (GO-T) and the degree of myocardial infarction has been confirmed by a more thorough study in this laboratory (Russell et al., 1960). This relationship exists in spite of the two main factors affecting the level of enzyme activity at any time. These factors include: 1) the rate of increase in enzyme activity; and 2) its rate of decay. The discovery of the changes in the activity of GO-T led to the realization that other enzyme activities are altered following coronary occlusion. Among
these are: 1) lactic dehydrogenase, LDH (Stewart and Warburton, 1961); 2) glutamic pyruvic transaminase, GP-T (Ruegsegger et al., 1959); 3) aldolase, ALD (Volk et al., 1956); and 4) acid phosphatase, ACP (Schoenfeld, 1963).

No attempt will be made to describe or discuss all of the various activities of all of these enzymes, but some of the characteristics consistent with all of them should be considered. Firstly, all of the enzymes mentioned above are present in heart muscle in varying concentrations and secondly, the activity of all of them increases in the blood plasma following coronary occlusion. The obvious inference is that these enzymes are released into the blood following coronary occlusion with subsequent tissue necrosis.

Recent investigations by Wroblewski (1963), Wroblewski and Gregory (1961), Warburton et al., (1963), Plummer and Wilkinson (1963) and others into the relative activities of the isoenzyme of LDH in normal tissue and plasma, and in the plasma during myocardial infarction, illustrating an increase in the activity in the plasma of the isoenzyme predominating in cardiac muscle, reinforces the theory of tissue necrosis as the prime factor effecting the increase in plasma enzyme activity following coronary occlusion.

All of the evidence, however, does not sustain this theory and, in fact, some appears to be directly contradictory. Schoenfeld (1963) has shown that acid phosphatase activity which is present in myocardial tissue in only small amounts, is greatly increased in the plasma of patients with myocardial infarction; furthermore, Hauss and Lepelman (1958) have found that the activities of other enzymes, tributyrinase and cholinesterase, are consistently decreased, instead of increased, in the plasma following coronary occlusion, and that the activity present in the
quantity of heart muscle does not appear to be sufficient to sustain the height of activity present in the plasma following coronary occlusion when the rate of disappearance of the enzymatic activity from the plasma is calculated. In reference to the latter of these statements, it has been shown by Hauss and Lepelman (1958) that maintenance of the level of activity of LDH commonly observed clinically and experimentally following coronary occlusion would require the necrosis of a quantity of myocardium far in excess of the weight of the entire heart. The weight of these and other observations has led Wroblewski (1963) to make the statement that, "...in experimental and clinical myocardial infarction, in addition to necrosis, other pathophysiological factors must be invoked to explain the serum enzyme alterations observed."

C. Lactic and Pyruvic Acids

The concentration of lactic acid in the ischemic myocardium by McGinty (1931) and Tennant et al. (1936) to be increased after coronary occlusion. Himwich et al. (1934) observed an increase in lactic acid in the coronary venous and arterial plasma after coronary occlusion. According to Bing (1954-55), the utilization of lactic acid by the cardiac musculature is decreased and often inverted following coronary embolization. The appearance of a negative extraction value for lactic acid presupposes the formation and liberation of lactic acid by the myocardium following coronary occlusion because the coronary venous concentration must be higher than the arterial level.

The inactivation of the citric acid cycle in the diffuse areas made ischemic by coronary embolization, accompanied by the continuance of the anaerobic portion of the glycolytic metabolic pathway, would thoroughly explain these phenomena.

The fate of pyruvate parallels that of lactate following coronary occlusion.
The change in pyruvate is less than that of lactate and may reflect differences in concentration or the anaerobic conversion of pyruvate to lactate by the ischemic musculature (Bing et al., 1954).

D. Glucose and Glycogen

No significant alterations have been previously reported in the plasma concentration or the extraction ratio of glucose following coronary occlusions although there is a tendency toward a slight increase in the latter parameter. Bing (1953) has expressed the belief that in the period immediately following coronary obstruction, glucose may be the primary metabolite, and that "apparently anaerobic glycolysis can still proceed in heart muscle under these conditions."

Glycogen disappears rapidly from the myocardial musculature following coronary occlusion, probably being converted to glucose and utilized as an energy source in the glycolytic pathway as a result of the loss of energy resulting from the suspension of the tricarboxylic acid cycle due to anoxia. The decrease in muscle glycogen and the increase in muscle lactic acid after coronary occlusion observed by Tennant et al. (1936) and the increase in lactic acid in the coronary venous blood reported by Himwich et al. (1934) are among many observations both chemical and histological, which support this conclusion.

E. The Catecholamines

The development of physiochemical techniques for the quantitative estimation of epinephrine and norepinephrine in biological fluids by von Euler and Floding (1955) and by Weil-Malherbe and Bone (1952) has enabled these and other investigators to more thoroughly analyze the alterations in the content of the catecholamines in various physiological and pathological situations.

Alterations in the concentration of the catecholamines have been
observed in both clinical and experimental cases of coronary occlusion. Gazes et al. (1959) reported an increase of both epinephrine and norepinephrine concentrations in the plasma of patients during the first 36 hour period of myocardial infarction. Experimental coronary occlusion performed on dogs by Richardson et al. (1960) resulted in an increase in circulating plasma norepinephrine concentration during the 24 - 36 hour period after coronary ligation.

Certain of the characteristics of the level of the catecholamines in the plasma require elaboration. One of these is the effect of the catecholamines, primarily 1-epinephrine, upon the metabolic activity of biological systems. There are numerous and varied, and while some of them (for example, the increases in the plasma concentrations of glucose and lactic acid) have been known for some time (Cori et al., 1930), others have been discovered more recently. Although methods of administration differ among the various investigations (single-dose and continuous intravenous infusion, subcutaneous injection, etc.), so that definite knowledge of the levels of the catecholamines in the plasma at all times are not known, and although the types of experimental animals differ among these investigations, it has been found convenient to class the effects together for the purposes of convenience and brevity and because of the similarity existing in the metabolic activity of the species concerned. Table I presents a list of the known effects of epinephrine upon the concentrations of some of the plasma components.

The effect of alterations in the pH of the blood on the level of concentration of catecholamines in the plasma has been reported by Nahas et al. (1960), Ligou and Nahas (1960) and Morris and Millar (1962). The conclusions derived from these experiments was that a decrease in pH of the blood results in an increase in the level of the circulating plasma
TABLE I

THE EFFECTS OF EPINEPHRINE ON THE
CONCENTRATIONS OF SOME OF THE PLASMA CONSTITUENTS

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<td>2 a decrease in bicarbonate</td>
<td>Coulson and Hernandez (1964)</td>
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<td>3 an increase in uric acid</td>
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<td>4 an increase followed by a decrease in potassium</td>
<td>Coulson and Hernandez (1964)</td>
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<td></td>
<td>D' Silva (1949)</td>
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<tr>
<td>5 an increase in lactate</td>
<td>Cori et al. (1960)</td>
</tr>
<tr>
<td></td>
<td>Coulson and Hernandez (1964)</td>
</tr>
<tr>
<td>6 an increase in urea</td>
<td>Coulson and Hernandez (1964)</td>
</tr>
<tr>
<td>7 a decrease followed by an increase in organic phosphorous</td>
<td>Soskin et al. (1941)</td>
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<td></td>
<td>Coulson and Hernandez (1964)</td>
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catecholamines. The induced acidosis had the same effect whether it was produced by lactic acid or hydrochloric acid infusion, or by carbon dioxide accumulation.

II. Retrograde Coronary Arterial Blood

Mautz and Gregg (1937) discovered that when the coronary artery was occluded and catheterized distal to the occlusion, blood flowed backwards out of the catheter. This and further investigations of this blood flow led to the conclusion that the retrograde blood appeared to be arterial because of its color. Other investigators have reported seeing a difference in color between retrograde and arterial blood (Russell et al., 1961, b). The importance of such measurements is obvious, because if a difference exists between the retrograde and arterial blood, and if this difference could be measured, then some insight into the quantity of blood which traverses the capillary bed from sources other than the primary arterial supply to the regions affected by the occlusion would be attained.

The results of investigations into the effects of coronary occlusion on the composition of the plasma have been presented. In general, each of these studies has been done on only one or two parameters of plasma composition. Since the techniques employed by each investigator vary, not only in design but also in performance, the necessity for studying the effects of coronary occlusion on a large number of parameters in the same series of animals becomes apparent.

These studies were designed to gather information upon chemical changes in blood and heart muscle following occlusion of a coronary artery. Experiments have been organized in relation to the following points:
1. the changes in plasma composition after coronary occlusion
   a. in arterial plasma
   b. in retrograde plasma
      1) absolute
      2) opposed to arterial
2. the significance of these alterations
3. the relationship between changes in plasma composition and those of the myocardium
4. the nature of the retrograde blood regarding
   a. oxygen saturation
   b. rate
   c. pressure
5. the relationship of retrograde flow rate to the collateral circulation.
METHODS

I. Experimental and Surgical Procedures

A. Series I

This series consisted of 22 mongrel dogs treated in the following manner. Each was anesthetized with a dose of 30 mg/kg of sodium pentobarbital. Arterial blood pressure was recorded from a catheter in the femoral artery and blood samples were obtained from the same catheter. The femoral vein was catheterized for injection of drugs and for the return of whole blood when this was required. Artificial respiration was maintained via an intratracheal tube with a Harvard positive pressure respiratory apparatus. Needle electrodes were installed so that Lead II of the electrocardiogram could be recorded. The thorax was opened by incision on the left side in the fourth intercostal space, the lung retracted, and the heart suspended in a pericardial cradle, which was prepared by suturing the opened pericardium to the sides of the thoracic incision. The left anterior descending coronary artery was freed by blunt dissection at a point approximately one centimeter below the left atrial appendage in systole. Two ligatures of 2-0 silk were then passed beneath the artery at this point. Sodium heparin was administered intravenously in a dose equal to 5.0 mg/kg. When all was in readiness, the upper ligature was tied, thereby occluding the artery in one stage; and the artery was at once catheterized distally with a PE-160 polyethylene catheter for collecting and measuring the blood flowing from the artery in a retrograde direction.
B. Series II

This series consisted of 17 mongrel dogs treated in the same manner as was described for the previous series, except for the following additions. The retrograde catheter was now connected to a bubble flow meter (see section on physiological measurements) which in turn was connected to the femoral vein through a lucite block drilled to accommodate three catheters in the form of a T (see Figure 1). The third catheter was connected to a pressure transducer, so that by occluding the catheter on the venous side of the T connector the retrograde arterial pressure could be recorded, or by occluding the catheter on the retrograde side of the T connector the systemic venous pressure could be recorded. After occlusion, systemic arterial, venous and retrograde coronary arterial samples were drawn for measurement of oxygen saturation.

C. Adrenalectomy

Bilateral adrenalectomy was accomplished through a midline incision into the abdominal cavity. The adrenal glands were isolated by blunt dissection when possible, their circulatory connections ligated, and the whole gland completely excised. Special effort was made to excise the glands in one piece so that the possibility of small quantities of tissue remaining in situ was abolished. The difficulties of removing the adrenal glands without rupturing the adjacent blood vessels (the renal vein and the vena cava) were formidable and only two of the five dogs operated on were in satisfactory condition (adequate blood pressure, etc.) after completion of adrenalectomy to be subjected to coronary occlusion and subsequent procedures. These two dogs, after closure of the laparotomy, were handled in the same manner as those of Series I.

D. Intravenous Infusions

1. Epinephrine - Three dogs were infused intravenously with epinephrine
FIGURE I

THE ARRANGEMENT OF THE CORONARY CATHETER
AND THE BUBBLE FLOW METER

LA = Left atrial appendage
LV = Left ventricle
RV = Right ventricle
A = Air bubble entrance
B = Drilled lucite blocks
C = Tubing to pressure transducer
D = Catheter to femoral vein
Bubble Flow Meter
at varying concentrations in order to determine its effects on various blood plasma components. The infusions were administered through a femoral venous catheter. A control infusion of one hour duration with saline was followed immediately by a two hour infusion of 1-epinephrine bitartrate in saline at the same rate. Saline was again infused for one hour following cessation of the epinephrine infusion. Infusion rates in the three dogs were 0.05, 0.50, and 1.00 micrograms per kilogram per minute of 1-epinephrine respectively. Heart rate, systemic arterial pressure, and Lead II of the electrocardiogram were recorded as described in the section on physiological measurements.

2. Lactate - Lactic acid was titrated with sodium bicarbonate to a pH of 7.4. Because the dog metabolizes lactate at a rapid rate, an initial dose was given, followed by a maintaining dose at a constant infusion rate. The initial dose was an amount calculated to increase the concentration of lactate in the total body water by approximately ten millimoles per liter. The maintaining dose was designed to maintain the total body water at its new level of concentration. The administration of a maintaining dose of 6.0 mM/hour to the first dog did not result in attaining the desired end; therefore, the second dog was administered lactate at a rate of 18.2 mM/hour. This proved to be much more satisfactory because, although the concentration was still not held at a constant level, the rate of its descent was considerably reduced.

E. Reserpine Pre-treatment

Reserpine (serpasil) in water was administered intravenously in daily doses equivalent to 0.10 milligrams per kilogram for three days prior to the day on which the experiment was conducted. The experiment amounted to the procedure previously described in the section on Series I. The side effects of large doses of reserpine (diarrhea, excessive hypotension, etc.)
in the opinion of the author, lowered the value of the animal as an experimental subject and only one dog was treated in this fashion.

F. **Dibenzyline Pre-treatment**

Dibenzyline was administered to a dog at a dose of 2.0 mg/kg one hour prior to occlusion of the coronary artery to block adrenergic effects at peripheral effector endings. Subsequent procedures employed for the experiment were essentially as described for Series I.

G. **Physiological Measurements**

**Blood pressure** measurements were done using the pressure transducer Model number MK-IV and the graphic recorder system of the E & M Instrument Company, Incorporated (Physiograph).

**Electrocardiographic** recordings were done using the MK-III preamplifier and the graphic recorder system of the E & M Instrument Company, Incorporated (Physiograph).

**Retrograde blood flow** rates were measured by either one of the two methods. The first employed collection during a measured time interval with subsequent measurement of the volume. The second method utilized a bubble flow meter constructed by the author which consisted of the following: (1) a lucite block bored in the form of a T which accommodated the retrograde catheter, a side-arm catheter for the injection of small air bubbles, and the measuring catheter; (2) the measuring catheter made of a length of PE-205 polyethylene tubing whose volume was calibrated at 0, 0.5, 1.0, 1.5, and 2.0 milliliters; and (3) a bulls-eye spirit level. The whole was mounted on a sheet of black plastic with the measuring tubing forming a spiral. The time of movement of the air bubble between any two points of the meter was determined with the aid of a stopwatch. Knowing the volume and the time, the rate was easily calculated. The apparatus was calibrated by infusing a known volume at constant rate into
it. It was found to be reasonably accurate in the range necessary for this work. The advantages of the bubble flow meter over the collection method are that it enables one to determine even the slowest of flow rates with greater accuracy and to obtain more frequent determinations. Attempts to utilize the rotameter in these experiments proved fruitless because of the extreme slowness of the rates of flow and because of the formation of clots in the apparatus in spite of the apparent complete heparinization of the animal.

In those instances where heart rate was indicated it was measured either by manually counting the electrocardiographic tracing or directly with the cardiotachometer manufactured by the E & M Instrument Company, Incorporated (Physiograph).

Constant rate continuous intravenous infusions were done using the Palmer ram-type slow injection apparatus equipped with the appropriate size Luerlock syringe.

II. Chemical Analyses

Calcium was estimated titrimetrically using the commercial procedure of the Hach Chemical Company, Ames, Iowa, in which Calver II powder is the indicator. Total alkaline earths were determined by the Schwarzenbach and Biederman (1948) procedure using Eriochrome Black T as the indicator. Magnesium was determined by calculating the difference between the results from the two procedures.

Chloride was estimated by the method of Schales and Schales (1941) using mercuric nitrate and s-diphenyl carbazone indicator.

Potassium and sodium concentrations were determined on 1:50 dilutions of plasma using the lithium internal standard technique in the Baird flame photometer.
Lactate was determined using the method of Barker & Summerson (1941) in which the lactate is converted to acetaldehyde and reacted with p-hydroxydiphenyl to produce a violet color which is read in a Klett-Summerson colorimeter at 560 m\(\mu\).

Bicarbonate plasma concentrations were estimated with the Van Slyke (1919) titrimetric technique where the \(\text{CO}_2\) is released by the addition of standard HCl and the remaining HCl is back-titrated to the end point of a blank, using phenol red indicator.

Plasma urea was estimated by the Conway microdiffusion procedure (Conway, 1957) utilizing boric acid containing a mixed indicator of bromocresol green and methyl red.

For amino acid determinations, a 1:10 protein-free filtrate of plasma was made with ammonia-free 95% ethanol. A 0.2 ml sample of this filtrate was added to one ml each of 80% phenol and 0.02 mM per 100 ml pyridine. The mixture was placed in a boiling water bath and 0.2 ml of 5% ninhydrin was added. The reaction was allowed to stand for 5 minutes, then made up to 10 ml with 60% ethanol (by volume), and read in a Klett-Summerson colorimeter at 570 m\(\mu\) (Troll and Cannon, 1953).

The concentration of alanine in the plasma of retrograde and arterial blood samples obtained from seven dogs was determined with the microbiological technique using leuconostoc citrovorum ATCC #8081 and the reagents of the Difco Laboratories (Sauberlich and Bauman, 1949 and Steele et al., 1949).

Three samples of plasma from each of eleven dogs were selected for individual amino acid analysis. The three samples were: 1) an arterial control (obtained just prior to occlusion); 2) an arterial sample taken at 3 hours after occlusion; and 3) a retrograde sample obtained at 3 hours after occlusion. The eleven samples in each category were pooled.
separately and three 1:10 protein free-filtrates prepared with absolute ethanol. Three milliliters of each were then evaporated to dryness, taken up in a minimum volume of 0.1 N HCl and analyzed using the Technicon Amino Acid Analyzer.

Pyruvic acid was determined by mixing 0.2 ml of plasma, 1 ml of water and 1 ml of Sigma Color Reagent (2-4 dinitrophenylhydrazine) and allowing the mixture to stand for 20 minutes at room temperature. After 20 minutes, 10 ml of 0.4 N NaOH was added and the tubes were read in a Klett-Summerson colorimeter 5 minutes later at a wavelength of 500 m\(\mu\) (Coulson, 1963).

Lactic acid dehydrogenase (LDH) activity of plasma was determined using the standard "Determatube" procedure of the Worthington Biochemical Corporation*, in which the rate of conversion of lactic acid to pyruvic acid by the enzyme is estimated by measuring the change of the coenzyme DPN to DPNH with the Mac Alaster-Bicknel Coenzometer.

Serum glutamic-oxaloacetic transaminase (SGOT) activity was estimated by a commercial procedure utilizing the kit supplied by the Sigma Chemical Company**, in which the rate of the reaction(\(\alpha\)-Ketoglutarate + L-Aspartate \(\xrightarrow{SGOT}\) Glutamate + Oxaloacetate) is determined by estimating the increased concentration of oxaloacetic colorimetrically at 500 m\(\mu\).

Glucose was determined with the commercial "Glucostat" reagents prepared by the Worthington Biochemical Corporation***, in which the enzyme glucoseoxidase catalyzes the conversion of glucose to hydrogen peroxide. Peroxidase catalyzes the reaction of hydrogen peroxide + reduced

*Worthington Biochemical Corporation, Freehold, New Jersey.
**Sigma Chemical Company, St. Louis, Missouri.
***Worthington Biochemical Corporation, Freehold, New Jersey.
chromogen to oxidized chromogen. The color of the oxidized chromogen is read in the Klett-Summerson colorimeter at 400 m\(\mu\).

Blood samples obtained from dogs in Series II were analyzed for the percentage of oxygen saturation using the oximeter model #10800 manufactured by the American Optical Company, Buffalo, New York.
RESULTS

I. Series I

A. Alterations in the Composition of the Plasma After Coronary Occlusion

1. Lactic Acid

The plasma of retrograde and systemic arterial blood samples obtained from 18 dogs following coronary occlusion was analyzed for the lactic acid. Seventeen of the eighteen dogs showed a moderate to marked increase in the plasma lactate concentration of both retrograde and systemic blood samples following coronary artery ligation. The remaining dog exhibited little alteration in plasma lactic acid concentration. The response was quite variable in magnitude, duration and time of onset with increases in magnitude ranging from a modest 10 - 15% to as much as 310% of the initial concentration. The mean systemic concentration achieved a maximum of 152% of the initial by 2 hours after occlusion (see Figure 2). Calculation of the average slopes of the curves resulted in values ranging from 1.5 to 155 %change/hour, so that some curves rose very steeply while others rose more slowly. The curves frequently show a dual response, i.e., an initial increase followed by a period of stable or decreasing concentration, which in turn was followed by a second increase. The period between the two increases varied from 15 minutes in one case to several hours in another.

2. Total Amino Acids

The plasma of retrograde and systemic arterial blood samples taken...
FIGURE 2
CHANGES IN THE CONCENTRATION OF LACTIC ACID IN THE PLASMA FOLLOWING CORONARY OCCLUSION

----- = Arterial
-------- = Retrograde
Lactate, % of Initial

Hours After Occlusion
from 15 dogs after coronary artery ligation was analyzed for the concentration of total amino acids. The concentration in both retrograde and arterial specimens increased consistently in all animals following coronary occlusion by as much as 325% of the initial concentration. The mean maximum arterial concentration equaled 133% of the initial at 3 hours after occlusion, while the concentration of the retrograde blood plasma attained a maximum mean concentration of 127% of the initial at 4 hours after occlusion (see Figure 3). The variation in rate of rise observed in the lactic acid curves was also present in plots of amino acid concentration against time, so that there was considerable variation in the duration of the response. Some curves attained maximum concentration and returned toward the control level during the first hour after ligation, while others rose more slowly and were still increasing up to 9 hours after occlusion. Frequently, especially in those curves with the lesser slopes, the increase was preceded by a slight decline during the first half-hour after occlusion. This event was more marked in the retrograde than in the arterial samples.

3. Individual Amino Acid Analysis

Three samples of plasma from eleven dogs were selected for analysis with the Technicon Amino Acid Analyzer. These three samples consisted of: 1) a systemic arterial control; 2) a systemic arterial sample taken three hours after occlusion; and 3) a retrograde sample taken three hours after occlusion. Calculation of the total amino acid concentration by summing the areas under the individual amino acid curves (excluding ammonia) demonstrated an increase in sample 2 to 143% of the control and an increase in sample 3 to 126% of the control (control, 2.330 mM/L; sample 2, 2.954 mM/L; and sample 3, 3.344 mM/L). No significant difference in composition could be determined between the three samples whatsoever.
FIGURE 3

CHANGES IN TOTAL AMINO ACID CONCENTRATION OF THE
PLASMA FOLLOWING CORONARY OCCLUSION EXPRESSED AS
PERCENT OF THE INITIAL CONCENTRATION

----- = Arterial
------- = Retrograde
The percent increase in total amino acids concentration agreed favorably with the value obtained by total amino acid analysis using the Ninhydrin Method.

4. Alanine

The plasma taken from ten dogs following coronary occlusion was analyzed microbiologically for the concentration of alanine. Plots of alanine concentration against time after occlusion demonstrated a marked increase in plasma alanine concentration. The increases varied considerably between dogs. The smallest increase was to 170% of the initial concentration at three hours after occlusion, and the greatest to 1500% of the initial at two hours after occlusion. One dog exhibited a 'moderate' rise to 200% during the first three to four hours and then increased dramatically from this value at four and one-half hours to 2700% at nine hours after occlusion. Increases in the plasma of the other dogs were distributed between these high and low values with concomittant distribution of rates of rise.

The concentration of alanine in the plasma was plotted against the concentrations of total amino acid on scales according to their respective proportions in normal plasma (alanine contributes approximately twenty percent to the normal total amino acid concentration). The results do not indicate a consistent disproportionate increase in alanine concentration when compared to the increases observed in the concentration of the total amino acids (see Figure 5).

5. Glucose

The plasma glucose concentration of systemic arterial and retrograde blood samples taken from seventeen dogs after coronary occlusion was estimated by the method previously described. The mean arterial concentration increased slightly at fifteen minutes, returned to the origin at
FIGURE 4

INDIVIDUAL AMINO ACIDS AS PERCENT OF THE TOTAL

Black  = Control arterial
Stippled = Arterial at 3 hours after occlusion
Striped  = Retrograde at 3 hours after occlusion
Amino Acids as % of Total

Glu(NH$_2$) 10
Asp(NH$_2$) 10
Thr 5
Val 5
Ser 5
Leu 5
Gly 5
Lys 5
Glu 5

Ala 25
Pro 20
Phe 15
Ileu 15
His 15
Tyr 15
Cy$_2$S$_2$ 15
Try 15
Citrl 15

Arg 10
Asp 10
HomoSer 10
Sarcosine 10
Met 10
Canavanine 10
3OH-His 10
Carnosine 10
α-amBut 10
OH-Pro 10
Orn 10

Unk Acid 5+6 10
Acid 3 10
Acid 1 10
Acid 2 10
FIGURE 5
THE RELATIONSHIP BETWEEN ALANINE AND TOTAL AMINO ACID CONCENTRATIONS IN THE SAME SAMPLES
Alanine, mmoles/liter

Amino Acids, mmoles/liter
one-half hour and then increased to 110% of the initial at two hours after occlusion. The level decreased to 102% at three hours and again increased to 134% of the initial at four hours after occlusion. The mean retrograde concentration followed a similar course, but achieved and maintained a higher concentration than did the arterial. At fifteen minutes after occlusion, the concentration was 125% of the initial. It then declined to 113% at one hour and then remained five to twelve percent above the arterial concentration (see Figure 6).

6. Calcium and Magnesium

The analysis of calcium and magnesium was performed on the retrograde and arterial plasma of nine dogs following occlusion. Eight out of the nine dogs analyzed exhibited a decrease in the concentration of magnesium in the retrograde plasma during the first hour after ligation. This decline was transient and the concentration then increased above the control level by two to three hours after occlusion. The plasma of seven of the nine dogs analyzed showed an increase in calcium concentration during the first hour. The concentration then returned toward normal and then again increased to still higher levels by two to three hours after occlusion.

7. Bicarbonate

The plasma of retrograde and systemic arterial blood samples taken after coronary occlusion was analyzed for bicarbonate concentration. These concentrations were plotted on the abcissa against the lactate concentrations on the ordinate. The pattern of these points presented in Figure 7 clearly demonstrate the inverse relationship existing between lactate and bicarbonate concentrations. The minimum bicarbonate concentrations obtained ranged from 2 - 6 mM/L.
FIGURE 6
CHANGES IN THE GLUCOSE CONCENTRATION OF THE PLASMA FOLLOWING CORONARY OCCLUSION

——— = Arterial
——— = Retrograde
FIGURE 7
THE RELATIONSHIP BETWEEN LACTATE AND BICARBONATE
CONCENTRATIONS IN THE SAME SAMPLES
8. Chloride

The analysis of the plasma chloride concentration of three dogs following coronary occlusion indicated little change in this parameter either in the retrograde or arterial samples. The absence of change in the chloride concentration showed that there was no shift in the distribution of the body water.

9. Pyruvic Acid

Analysis of pyruvic acid in the plasma following coronary occlusion demonstrated an increase which paralleled the changes in lactate concentration (see Figure 8). The changes, although small in concentration, were relatively large when computed as percent of the initial concentration. (A change in one dog from 0.25 to 0.48 mM/L amounts to an increase to 192% of the initial concentration).

10. Enzymes:
   a. Lactic Dehydrogenase (LDH)

The plasma of retrograde and systemic arterial blood samples taken from eighteen dogs after coronary occlusion was analyzed for LDH activity by the method previously described. A graph of the mean activities of plasma LDH against time after occlusion demonstrated an increase in activity beginning almost immediately after ligation. At fifteen minutes after occlusion the mean activity of both retrograde and systemic arterial plasma had increased by ten percent. At one-half hour the retrograde plasma activity increased by fifty percent and the arterial by twenty percent. The mean systemic arterial plasma activity increased to a maximum value of 234 percent of the initial at three hours after occlusion, while the retrograde plasma had a mean activity equal to 352% of the initial (Figure 9).
FIGURE 8
THE RELATIONSHIP BETWEEN PYRUVATE AND LACTATE
CONCENTRATIONS IN THE SAME SAMPLES
FIGURE 9

CHANGES IN LACTIC DEHYDROGENASE (LDH) ACTIVITY OF THE PLASMA FOLLOWING CORONARY OCCLUSION

——— = Arterial

——— = Retrograde
b. **Glutamic Oxaloacetic Transaminase (GO-T)**

The plasma of retrograde and systemic arterial blood samples taken from three dogs were analyzed for glutamic oxaloacetic transaminase (GO-T) activity. A plot of these activities against the LDH activities in the same samples (see Figure 10) demonstrates a direct linear relationship between the two enzyme activities. The samples high in LDH activity were also high in GO-T activity and vice versa.

B. **Retrograde Blood Flow Rate**

The retrograde blood flow rate was determined in seventeen dogs after coronary occlusion. The flow rates ranged from 0.15 - 4.85 ml/min. The retrograde blood flow rate was determined in eleven of the seventeen dogs continuously for at least four hours after occlusion. The averages at periodic intervals were determined and plotted against time and are represented by the curve in Figure 11. The flow rates showed an increase during the first hour after occlusion and then fell to a lower more stable level. Observations were continued in some dogs for periods of up to eleven hours after occlusion and the rates were seen to either remain at the three to four hour level or exhibit a very gradual decline. The transient alterations in retrograde flow rate are not paralleled by like changes in arterial blood pressure. This can be clearly seen in Figure 12 in which the retrograde blood flow rate and arterial blood pressure are plotted. Later after occlusion the retrograde flow rate and arterial pressure appear to coincide. An explanation of the early increase in retrograde flow rate may lie in the early ability of the collateral vessels to respond to the hypoxia with dilatation. Later, after the damage becomes more extensive, they become less responsive and act more as mere conduits, thus more nearly reflecting changes in arterial pressure.
FIGURE 10
THE RELATIONSHIP BETWEEN LACTIC DEHYDROGENASE (LDH) AND GLUTAMIC OXALOACETIC TRANSAMINASE (GO-T) ACTIVITIES IN THE SAME SAMPLES
A scatter plot showing the relationship between LDH units/ml and GO-T units/ml.
FIGURE 11

CHANGES IN RETROGRADE BLOOD FLOW RATE
FIGURE 12
A COMPARISON OF CHANGES IN RETROGRADE BLOOD FLOW RATE AND ARTERIAL BLOOD PRESSURE IN DOG NUMBER 16 OF SERIES I.

FAP = Femoral arterial pressure
s = systolic
d = diastolic
II. Series II

A. Oxygen Saturation

Retrograde, systemic arterial and systemic venous blood samples were drawn from thirteen dogs during the first half-hour after coronary occlusion and analyzed for the percentage of oxygen saturation. The mean oxygen percent saturation of retrograde blood was 90% (range 69 - 99), the systemic arterial was 91% (range 75.5 - 99) and the systemic venous was 57% (range 36 - 84) (see Table 2). Subsequent analysis of the retrograde and arterial blood oxygen saturation during the next several hours after occlusion failed to detect any measurable difference from the values initially obtained.

B. Retrograde Blood Flow Rate

The mean initial retrograde blood flow rate was 1.014 (range 0.3 - 2.5) ml/min. The pattern of change observed in the rate of flow of retrograde blood after coronary occlusion was similar to that observed in Series I.

C. Blood Pressure Measurements

The mean retrograde pressure obtained from measurements on ten dogs was 22.7 mm of Hg (range 16 - 30). The mean venous pressure in five of these dogs was 6.1 mm of Hg (range 2 - 10). The mean retrograde pressure when measured as it flowed into the femoral vein was 8.3 mm of Hg (range 6 - 11). The mean arterial blood pressure at the time of the retrograde pressure measurements was 103/63 mm of Hg (range 70-140/40-110). The mean retrograde flow rate in these ten dogs was 1.05 ml/min (range 0.3 - 2.5) (see Table 3). The retrograde pressure was fairly constant throughout the experiment; not varying by more than 1 - 2 mm of Hg, except when the arterial pressure changed suddenly or during periods of ectopic activity. The retrograde pressure followed alterations in the arterial pressure, but often lagged behind the arterial
<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARTERIAL, VENOUS AND RETROGRADE OXYGEN SATURATION, ARTERIAL BLOOD PRESSURE AND RETROGRADE FLOW RATE</td>
</tr>
<tr>
<td>Dog No</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Art.</td>
</tr>
<tr>
<td>Ven.</td>
</tr>
<tr>
<td>Retro.</td>
</tr>
<tr>
<td>Sys.</td>
</tr>
<tr>
<td>Diast.</td>
</tr>
<tr>
<td>Retrograde Flow, ml/min</td>
</tr>
</tbody>
</table>
**TABLE 3**

**RETROGRADE, SYSTEMIC ARTERIAL AND VENOUS PRESSURES AND RETROGRADE FLOW RATE OF DOGS IN SERIES II**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP</td>
<td>Retrograde pressure</td>
</tr>
<tr>
<td>RPO</td>
<td>Retrograde pressure open to the vein</td>
</tr>
<tr>
<td>FVP</td>
<td>Femoral venous pressure</td>
</tr>
<tr>
<td>FAPs</td>
<td>Systolic femoral arterial pressure</td>
</tr>
<tr>
<td>FAPd</td>
<td>Diastolic femoral arterial pressure</td>
</tr>
</tbody>
</table>

*(All pressures expressed as mm of Hg)*

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>Retrograde flow rate in ml/min</td>
</tr>
</tbody>
</table>

52
<table>
<thead>
<tr>
<th>DogNo</th>
<th>RP</th>
<th>RPO</th>
<th>FVP</th>
<th>FAPs</th>
<th>FAPd</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>25.0</td>
<td>6.0</td>
<td>—</td>
<td>130</td>
<td>90</td>
<td>2.1</td>
</tr>
<tr>
<td>5</td>
<td>25.0</td>
<td>11.0</td>
<td>10.0</td>
<td>140</td>
<td>110</td>
<td>0.3</td>
</tr>
<tr>
<td>7</td>
<td>30.0</td>
<td>10.0</td>
<td>—</td>
<td>90</td>
<td>55</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>18.5</td>
<td>7.5</td>
<td>2.0</td>
<td>115</td>
<td>85</td>
<td>0.6</td>
</tr>
<tr>
<td>10</td>
<td>22.0</td>
<td>7.0</td>
<td>6.0</td>
<td>90</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>11</td>
<td>24.0</td>
<td>8.5</td>
<td>6.0</td>
<td>70</td>
<td>40</td>
<td>1.5</td>
</tr>
<tr>
<td>12</td>
<td>22.5</td>
<td>9.0</td>
<td>6.5</td>
<td>90</td>
<td>40</td>
<td>0.5</td>
</tr>
<tr>
<td>15</td>
<td>20.0</td>
<td>6.0</td>
<td>—</td>
<td>105</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>17</td>
<td>24.0</td>
<td>10.0</td>
<td>—</td>
<td>100</td>
<td>55</td>
<td>1.5</td>
</tr>
<tr>
<td>Mean</td>
<td>23.4</td>
<td>8.3</td>
<td>6.1</td>
<td>103</td>
<td>63</td>
<td>1.1</td>
</tr>
<tr>
<td>S.D.</td>
<td>3.3</td>
<td>1.8</td>
<td>2.5</td>
<td>21</td>
<td>23</td>
<td>0.8</td>
</tr>
</tbody>
</table>
pressure change by as much as 2 - 2.5 seconds.

D. Sodium and Potassium Concentration of Heart Muscle

Three of the seventeen dogs of this series were kept alive for a period of 24 hours after occlusion. The ventricles of these dogs were divided into two sections: 1) normal and 2) normal plus infarct. After complete digestion with concentrated nitric acid and dilution with water and standard lithium they were analyzed for their sodium and potassium content. The sodium gained and potassium lost by the infarcted tissue were then calculated into meq/kg wet weight of tissue. These results, along with the average retrograde blood flows during the first several hours after occlusion, are presented in Table 4.

III. Intravenous Infusions

A. Epinephrine

The intravenous infusion of increasing amounts of 1-epinephrine produced concomitant increases in the plasma concentrations of lactate and glucose (see Figure 13). The alterations in the concentrations of the total amino acids in the plasma were not quite as consistent and the increases were preceded by a period of initial depression (see Figure 14). The infusion of 1-epinephrine at the two lower rates produced little change in the plasma LDH activity. However, infusion at the rate of 1.0 \( \mu \text{g/kg/min} \) increased the LDH activity from a normal of 67 units/ml to 339 units/ml. The activity continued to increase to 352 units/ml even after the infusion had been stopped for one-half hour (see Figure 15).

No significant alteration in the systemic arterial blood pressure or heart rate could be observed during the periods of infusion of 1-epinephrine. Very little change in any of the metabolic parameters was noted during the periods of control saline infusion.
TABLE 4

SODIUM AND POTASSIUM SHIFTS AND RETROGRADE FLOW RATES OF DOGS IN SERIES II
<table>
<thead>
<tr>
<th>Dog No.</th>
<th>7</th>
<th>11</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na Gain*</td>
<td>6.23</td>
<td>4.45</td>
<td>2.5</td>
</tr>
<tr>
<td>K Loss*</td>
<td>5.90</td>
<td>2.91</td>
<td>1.5</td>
</tr>
<tr>
<td>Retrograde Blood Flow†</td>
<td>10.20</td>
<td>6.78</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* meq/kg wet wt of tissue
† ml/ml/min
FIGURE 13

CHANGES IN LACTIC DEHYDROGENASE (LDH) IN THE ARTERIAL PLASMA DURING AND FOLLOWING THE INTRAVENOUS INFUSION OF 1-EPINEPHRINE (0.001 mg/kg/min)
FIGURE 14

CHANGES IN THE CONCENTRATIONS OF GLUCOSE AND LACTIC ACID OF THE ARTERIAL PLASMA DURING AND FOLLOWING THE INTRAVENOUS INFUSION OF 1-EPINEPHRINE

--- = Glucose
--- = Lactate

■ = 0.001 mg/kg/min
▲ = 0.0005 mg/kg/min
● = 0.00005 mg/kg/min
FIGURE 15

CHANGES IN THE CONCENTRATION OF THE TOTAL AMINO ACIDS
OF THE PLASMA DURING AND FOLLOWING
THE INFUSION OF 1-EPINEPHRINE

■ = 0.001 mg/kg/min
▲ = 0.0005 mg/kg/min
• = 0.00005 mg/kg/min
B. Lactate

No alteration in the plasma concentration of glucose, total amino acids or LDH were observed during, and one-half hour following the cessation of, two hours of continuous infusion of lactate. During the period of infusion, the plasma lactate concentration increased to 24.2 and 21.9 mM/L in two dogs, respectively (see Figure 16).

IV. Adrenalectomy

Analysis of the plasma of blood samples taken from two adrenalectomized dogs following coronary occlusion yielded the following results. An increase in total amino acid plasma concentration was present in both dogs (AD-1 increased to 127% and AD-2 to 122% of their respective initial concentrations). There was an increase in the plasma lactic acid concentration in both dogs (AD-1 increased to 129% and AD-2 to 150% of their respective initial concentrations). The concentration of glucose in the plasma increased tremendously in the first dog to 859% of the initial concentration, while the second dog exhibited little, if any, alteration in this parameter. LDH activity also increased in the first dog, rising to 315 units/ml from a control of 55 in the first hour after occlusion. The activity then fell toward normal to 85 units/ml, remained near this level for the third hour and increased again to 325 units/ml by the end of the fourth hour after occlusion. The plasma activity of the second dog exhibited little change in this parameter.

The retrograde blood flow rate was relatively low, possibly due to the relatively low arterial blood pressure. During the first two hours after occlusion, the flow in dog number one was 0.05 ml/min. It increased to 0.16 ml/min during the second hour and to 0.20 ml/min during the next 45 minutes. The flow rate in the second-dog was more constant, beginning
FIGURE 16
CHANGES IN THE LACTATE CONCENTRATION OF THE
ARTERIAL PLASMA DURING AND FOLLOWING THE
INTRAVENOUS INFUSION OF SODIUM LACTATE

----- = Dog 1
-------- = Dog 2
at 0.22 ml/min, increasing to a maximum of 0.30 ml/min toward the end of the first hour and decreasing to 0.17 ml/min by the end of the second hour after occlusion.

V. Dibenzyline Pre-treatment

Analysis of the plasma of blood samples following coronary occlusion in the dog pre-treated with dibenzyline demonstrated diminished changes in certain parameters and inconsistencies in others. The glucose concentration was initially high (150.9 mg%), but exhibited little alteration after occlusion (maximum increase was 11% at three hours). The lactate concentration increased to 130% of the initial by three hours after occlusion, after an initial decline to 83%. The amino acid concentration, after an initial decline to 86%, returned to the control level by three hours after occlusion. The LDH activity was very low initially and remained so throughout the period after occlusion (range 21 - 36 units/ml).

The retrograde blood flow rate ranged from 0.63 ml/min at eighteen minutes after occlusion to 0.36 ml/min after three hours. The oxygen percent saturation of the arterial blood was determined before and after coronary occlusion. There was no change from the control value of 97% up to two hours after occlusion. The arterial blood pressure went into periodic alterations after the administration of dibenzyline. The pulse pressure appeared abnormally low following thoracotomy (85/75 mm of Hg) and did not improve throughout the remainder of the experiment. The mean arterial pressure varied little during this time.

An unusual amount of ectopic activity was observed following coronary ligation. It had diminished, but still remained, three hours after occlusion.
VI. Reserpine Pre-treatment

Analysis of the plasma of blood samples taken from a dog pre-treated with reserpine after coronary occlusion yielded the following results. The total amino acid concentration increased slightly from the control level (maximum increase to 118% of the initial concentration). The lactic acid concentration exhibited little alteration during the first two hours after occlusion, except for an initial decline, but reached a level of 180% at three hours and 196% at five hours after occlusion. The glucose concentration increased gradually after a very brief decline (during the first half-hour after occlusion) and reached a level of 193% of the initial at five hours. The LDH activity was initially relatively low (13 units/ml) and remained low for the duration of the experiment, never exceeding 40 units/ml.

The retrograde blood flow rate was initially high (1.27 ml/min) despite the relatively low arterial blood pressure 70/45 mm of Hg). It slowly decreased to 0.22 ml/min by four hours after occlusion and appeared to decline with the constantly falling blood pressure. The percent oxygen saturation of the retrograde blood was the same as the arterial (92%) and the venous was 42%.
FIGURE 17
CHANGES IN LACTIC ACID CONCENTRATION OF THE ARTERIAL PLASMA FOLLOWING CORONARY OCCLUSION

■ = Dibenzyline pretreatment
▲ = Adrenalectomized
● = Reserpine pretreatment
Lactate, % of Initial

Lactate, % of Initial

Hours After Occlusion

100
110
120
130
140
150
160
170
180
190
200
FIGURE 18

CHANGES IN GLUCOSE CONCENTRATION OF THE ARTERIAL PLASMA FOLLOWING CORONARY OCCLUSION

■ = Dibenzyline pretreatment
▲ = Adrenalectomized
○ = Reserpine pretreatment
FIGURE 19

CHANGES IN THE LACTIC DEHYDROGENASE ACTIVITY OF THE ARTERIAL PLASMA FOLLOWING CORONARY OCCLUSION IN ADRENALECTOMIZED DOG NUMBER 1
DISCUSSION

I. Alterations in Plasma Composition After Coronary Occlusion

Certain aspects of each of the analyses performed require some individual attention. Each analysis, therefore, will be discussed individually and an attempt made to correlate the results of these analyses into a general scheme.

A. Lactic Acid

The increase in lactic acid observed following coronary occlusion might be attributed to the leakage of this substance from the ischemic portion of the myocardial tissue. Calculation of the amount present in the total extracellular fluid volume however, apparently disproves this theory as a complete explanation of the magnitude of most of the responses observed. It can be assumed that most of the lactate produced in the deprived region of the heart during complete ischemia is the result of anaerobic metabolism of glucose derived from the available glycogen in the heart muscle. It has been reported by Cruickshank and Shrivastava (1930) and by Merrick and Meyer (1954) that the normal dog ventricle contains approximately 0.6% glycogen. Under the conditions of the methods employed in this study, about fifteen grams of ventricular myocardium can be expected to be completely ischemic in an average ten kilogram dog. This, then, would presuppose the liberation of approximately 100 milligrams of glucose (15 grams of tissue x 0.0006 grams of glucose/gram of tissue x 1.11, factor for the hydration of glucose from glycogen) which may be assumed to be converted to the same quantity of lactate. On the other hand,
an increase in plasma lactate concentration of only one millimole/liter in a ten kilogram dog would require the addition of a minimum of 200 milligrams of lactate making the following assumptions: 1) that the lactate enters only the extracellular fluid volume; 2) that there is no utilization of lactate during this period; and 3) that there is no elimination of lactate by any means during this period. In other words, a moderate increase in plasma lactate concentration, and one that is often observed following coronary occlusion, would require, at the very least, the complete disruption of twice as much myocardial tissue as normally results from this type of preparation. Therefore, an additional source of lactate must be found to explain this phenomenon. However, this does not presuppose that the lactate elaborated by the anoxic myocardium does not contribute to the increased plasma concentration. The increase in lactate concentration of the retrograde plasma over that of the arterial plasma at 62% of the simultaneous points on the curves compared, indicates a tendency for the lactate to diffuse from the ischemic myocardium into the general circulation. It seems clear, however, that an important fraction of the lactate liberated must be from other sources.

B. **Amino Acids**

The increase in amino acid concentration in the retrograde and arterial plasma following coronary occlusion may, or may not, be directly related to tissue necrosis. No analogy can be made between the protein content of the tissue and the amount of increase in plasma amino acid concentration as was done for lactic acid because the number of amino acids present in a quantity of protein cannot be so precisely calculated. The exact amino acid composition of each protein fraction would have to be known in order to do this.

However, if the increase observed was due to tissue necrosis, it would
be expected that there would be a disproportionate increase in glutamic acid, since the muscle is rich in this amino acid. However, no such increase could be observed in the analysis of the individual amino acids, either in concentration per se, or as a percentage of the total amino acid content. Rather, there was an increase in all of the constituents. In addition, it has been shown by Chanin et al. (1956) that the amino acid content of the ischemic myocardium does not begin to diminish until 8 - 16 hours after ligation of the coronary artery.

The differences observed between retrograde and arterial plasma may indicate the utilization of amino acids by the ischemic myocardium as a source of available energy. Out of 144 simultaneous points exhibiting a difference between retrograde and arterial plasma concentration, 68% of the retrograde concentrations were lower than the arterial. These results are supported by the findings of Bing et al. (1954) which demonstrated a positive amino acid extraction ratio in normal human hearts.

C. Glucose

The alterations in the plasma concentration of glucose were periodic, exhibiting rising and falling phases during the first four hours after occlusion. The differences between animals were also more variable then those of the other parameters. The majority of dogs (13 out of 18) exhibited a general increase in plasma glucose concentration while two showed little change and three an overall decline. However, of the five that exhibited a general decline, or little overall change, all exhibited an early increase. The pattern of alteration in these dogs appear to be similar to the effects of norepinephrine as reported by Tanaka (1956) and may represent differences in proportions of the two catecholamines released following coronary occlusion.

The early increase in retrograde glucose concentration indicates the
diffusion of glucose from the heart muscle during the first hour after occlusion. The exact nature of this diffusion cannot be ascertained since it would be assumed that any glucose elaborated from the breakdown of muscle glycogen stores would be anaerobically metabolized by the tissues. The quantity of glucose emanating from the retrograde catheter could theoretically come from the ischemic heart muscle because the amount of muscle devoid of glycogen following occlusion contains enough latent glucose to account for the difference. For example, dog number fifteen contained an excess of approximately fifteen milligrams in the retrograde plasma during the first hour after occlusion, which amount could be elaborated from the glycogen present in 2.30 grams of ventricular myocardium. Since the amount of muscle destroyed by the method employed is usually 6 - 7 times as much, it is possible that the excess glucose is emanating from myocardial glycogen stores.

D. LDH and GO-T

The early increase in enzyme activity does not conform with the majority of reports of investigations regarding increased activity following coronary occlusion. Early increases in enzyme activity have been reported by Bing et al. (1956), but no significance was attached to this observation.

The differences in the mean enzyme activities of the retrograde and arterial plasma were not supported by a study of the frequency of occurrence of an increase in retrograde plasma activity over arterial. This occurred in only 52% of the 149 points compared during the first three hours after occlusion.

E. Calcium and Magnesium

The decrease in magnesium observed during the first hour after occlusion cannot be explained by leakage of electrolyte from necrotic tissue since the intracellular concentration of magnesium is higher than the extracellular.
The increase in calcium and magnesium may be attributed, however, to the progressive acidosis produced by the increasing lactic acid concentration. A plot of calcium and magnesium concentrations against lactate concentration indicates a direct relationship between the two parameters above a lactate concentration of ten millimoles per liter. There appears to be no correlation between the two parameters below this level (see Figure 20).

F. Retrograde/Arterial Concentration Differences

The differences observed between the composition of retrograde and arterial plasma has raised the question of whether the differences are real, or are the result of some artifact.

Evidence that the differences are real include the following considerations: 1) when simultaneous points are compared for differences between retrograde and arterial plasma concentrations approximately two thirds of the points are in one direction or the other (except for LDH); and 2) differences often are consistent in the same dogs (see Figure 21).

On the other hand, the suspicion may exist that the differences are due to water absorption by the ischemic myocardium. It has been shown by Russell et al. (1961) that by four and one-half hours after occlusion, the ischemic tissue increases its water content from 77.5 to 80.6 percent, or an increase in water content of 0.031 ml per gram of tissue. In a fifteen gram infarct this would amount to 0.465 ml of water absorbed from the circulation in this period. Assuming that the rate of water absorption is linear during this interval, the rate of increase in water content of the tissue concerned would amount to 0.00172 ml/min. If we assume that all of this water is supplied to this tissue by the measured retrograde blood flow, and if we assume a very slow flow rate of 0.1 ml/min (0.06 ml of plasma assuming a 40% hematocrit), then the retrograde plasma would
FIGURE 20

The relationship between the concentrations of the total alkaline earths (calcium and magnesium) and lactate in the same samples.
FIGURE 21

CHANGES IN THE TOTAL AMINO ACID CONCENTRATION
OF THE PLASMA FOLLOWING CORONARY OCCLUSION

_______ = Arterial

-------- = Retrograde

Each symbol represents one experiment.
be concentrated by only 2.86%. Since the normal retrograde flows are in excess of the amount assumed, and since the observed differences are generally in excess of this percentage, it can be concluded that water absorption plays only a minor role in determining differences between retrograde and arterial plasma concentrations.

II. Significance of Alterations in Plasma Composition

The changes observed in the composition of the arterial and retrograde plasma may be summarized as follows:

1) an increase in lactate with a concomitant decrease in bicarbonate
2) an increase in total amino acids (including an increase in alanine)
3) an increase in glucose
4) an increase in LDH and GO-T
5) an increase in pyruvate
6) an increase in calcium
7) an alternating decrease and increase in magnesium
8) no change in chloride

The magnitude of the increase in lactic acid has been shown to be too great to be accomplished by leakage from damaged myocardium into the general circulation. Reference to the inability of the myocardium to supply all of the increased enzymatic activity found in the plasma has already been made in the introduction. In addition, the initial decrease in magnesium is in the wrong direction to emanate from this region. It does appear, however, since differences in lactic acid and glucose are found between retrograde and arterial plasma, that leakage from the ischemic myocardium probably is a contributing factor, with regard to these constituents. However,
this is not true for the amino acids since the retrograde plasma concentrations are generally higher than the arterial.

In light of these incongruities, another explanation is required to account for the observed alterations in plasma composition following coronary occlusion. Tissues other than the ischemic region of heart muscle must contribute to the changes by liberation and uptake of certain constituents. The catecholamines appear to be likely agents in evolving these more widespread effects.

The reasons for implicating the catecholamines are as follows: 1) it has been reported by Gazes et al. (1959) and by Richardson et al. (1960) that there is an increase in the circulating plasma catecholamine concentration following coronary occlusion; and 2) they are the only endogenous substances which have demonstrated the actions necessary to produce the observed changes in both magnitude and direction. The intravenous infusion of 1-epinephrine mimics certain effects of coronary occlusion: an increase in plasma lactate, glucose and LDH and an increase in total amino acids after an initial decline. The similarity between the effects of coronary occlusion and 1-epinephrine on the activity in the plasma of other enzymes has been reported by Maling et al. (1960) and by Highman et al. (1959). D' Silva (1949) has reported an increase in plasma potassium concentration followed by a subsequent decline below the control level after the administration of various catecholamines.

The great similarity between the effects of coronary occlusion and those of the catecholamines in so many parameters cannot be attributed to coincidence and, therefore, they must be related. It must be concluded that the occlusion of a major coronary artery is accompanied by a sympathomimetic response which produce certain of the alterations observed in the plasma composition.
III. Sources of Catecholamines and Nature of the Response

The first hypothetical source of circulating catecholamines to be considered is the release of the catecholamines stored in the myocardium itself. Recent investigation by Russell et al. (1961, a) has shown that the normal ventricular myocardium contains 0.018 and 0.830 μg/gram of epinephrine and norepinephrine respectively. Four and one-half hours after occlusion the ischemic myocardium contains 0.008 and 0.64 μg/gram representing a loss of 0.010 and 0.190 μg/gram of epinephrine and norepinephrine respectively. Assuming that the rate of loss is linear and assuming a mass of tissue of fifteen grams, the rate of release of epinephrine and norepinephrine into the general circulation would amount to 0.0039 μg/kg/min. This quantity is too low to account for the results observed. Only if they were released suddenly into the circulation would there be a possibility that they could exert some effect upon the plasma composition. In an attempt to establish the source of the catecholamines elaborated after coronary ligation, bilateral adrenalectomy was performed successfully on two dogs. The increase in LDH activity, along with the increased concentration of the other constituents in both dogs, following coronary occlusion eliminates the adrenal medulla as the only source of these endogenous materials. The failure to suppress the sympathomimetic response is in agreement with the work of Richardson et al. (1959) which showed that bilateral adrenalectomy did not abolish the increase in circulating catecholamines resulting from coronary occlusion.

The elaboration of the catecholamines which apparently cause the changes in the chemical composition of the plasma following coronary occlusion must stem from post-ganglionic nerve endings because the chemical changes can occur in spite of previous bilateral adrenalectomy and because no other important source of these hormones is known to exist.
The positive response exhibited by the reserpine pre-treated dog after coronary occlusion was inconclusive. According to Burn (1963), the catecholamine stores of the heart are depleted to zero with reserpine. However, other investigations have shown that the catecholamine stores of other tissues are only reduced by this drug. Although there may not be enough catecholamines present to elicit changes in vascular tone, the fact that metabolic alterations are elicited by quantities below those which initiate vascular responses, supports the possibility that enough remains to elicit the alterations observed after coronary occlusion. The unusually low activity of LDH in the plasma (13 units/ml) and the relatively low initial concentration of lactic acid (4.6 mM/L) demonstrate that the reserpine was not without some effect on the plasma composition.

Pre-treatment with dibenzyline (phenoxybenzamine hydrochloride), an adrenergic blocking agent, also produced a relatively low initial plasma LDH activity. The initial plasma lactic acid concentration, however, was within the range normally observed (7.1 mM/L). Although the response was not completely abolished by this drug, it appeared to be somewhat reduced. Further investigation with varying dosages of this drug, and other adrenergic blocking agents, may shed more light on the subject.

The infusion of sodium lactate was used to determine if the lactate ion itself had the effect of increasing the plasma LDH activity or any of the other parameters. Since extremely high lactate concentrations were achieved with only negligible changes in LDH activity and glucose and amino acid concentrations, it was concluded that the presence of the lactate ion, in itself, was not the cause of the changes observed in the other parameters. The infusion of free lactic acid, however, probably would have such an effect, because it has been shown that there is an increase in circulating catecholamines following the initiation of acidosis by this
procedure. This relationship may be important in these experiments because there is the possibility of the beginning of a cycle. The catecholamine response can increase the lactic acid resulting in severe acidosis which, in turn, can result in the elaboration of more catecholamines. The initiation of such a cycle would prove to be disastrous to the animal, leading ultimately to severe acidosis and death. It has been shown by Nahas et al. (1960) that the vascular effects of the catecholamines are reduced during acidosis, but this does not necessarily mean that the same is true of their metabolic effects which needs further investigation. Bicarbonate concentrations have been observed in the present experiments as low as 2.0 meq/L. Assuming no change in the carbonic acid fraction, the pH would be equal to the pK (6.1) plus the log of $\frac{2.0}{1.3}$, or about 6.3. This condition of extreme acidosis is incompatible with life and the death of the dog soon after this event can easily be attributed to this cause.

The question remains as to what exact mechanism triggers the release of catecholamines. The mechanism could be either of two types: humoral or neural. It may be initiated by some substance elaborated in and/or escaping from the area of ischemia, or a train of impulses may be set up along afferent pathways.

Regarding the humoral aspect, attempts to elicit excitatory activity from homogenates of infarcted myocardium infused into the coronary circulation have proven to be fruitless thus far. However, no such attempt has been made to elicit metabolic responses. Perhaps no such response exists, but the possibility persists until it has been tried. Cross-circulation experiments would prove of little value in this respect because the substances produced by the one dog would surely produce the same effects in the other without establishing the origin or mechanism of the response. Increased excitatory activity has sometimes been observed during periods
when the retrograde coronary catheter was occluded, suggesting that an excitatory substance may be elaborated in the myocardium during periods of ischemia. The high speed tachycardias observed did not occur when the catheter was open either to the outside or to the femoral vein. These occurrences were not common, however, and were observed in only two dogs.

The existence of afferent nerves from the heart have been established by Neil and Joels (1961) but their exact role in the regulation of the heart is not known. Reports in the literature of studies of the discharges of the cardiac afferents following coronary occlusion could not be found and this offers yet another area for future research. The obvious answer would be to test the chemical responses to coronary occlusion of dogs whose hearts were previously completely denervated. The absence of the chemical alterations after denervation would indicate that there was a neural pathway involved; whereas, if the response persisted, the neural aspect might be discounted.

IV. Oxygen Saturation of Retrograde Blood

The failure of the retrograde blood to exhibit any consistent difference in percent of oxygen saturation from that of arterial blood may be inconclusive. Because of the sigmoidal nature of the oxygen dissociation curve, the blood may experience a decrease in oxygen content without exhibiting a significant decrease in percent saturation. Measurement of the \( \text{pO}_2 \) of the retrograde blood may prove to be a more reliable criterion in this regard.

The differences between retrograde and arterial plasma concentrations observed in some constituents at times appear to be real. If this be so, then the arterial blood must either traverse the capillary bed or come in
contact with extracellular fluid proximal to the capillary areas in order to effect the transfer of such substances. It is reasonable to assume that oxygen would be more easily diffusible because it is a gas and because of the large gradient existing between the hypoxic tissue and the arterial blood.

V. Sodium and Potassium Concentration of Heart Muscle

The complete heparinization of the dog for the purposes of coronary catheterization and retrograde measurements was not a problem so long as the chest was open. However, it became a problem when attempts were made to close the thoracic incision and keep the animal alive for at least 24 hours. The dogs invariably died during the first three to four hours after closure. Of the first sixteen dogs operated on, only three survived longer than 18 hours, and only two as long as 24 hours after occlusion. Autopsy usually revealed a fairly large quantity of fluid in the chest cavity and the lungs appeared to be hemorrhagic. Death in these animals was believed to have resulted from the oozing of fluids into the chest cavity from the incision. The experience with the seventeenth dog appeared to support this supposition. In this dog, just prior to closing the incision, the excess heparin remaining in the animal was neutralized by the intravenous administration of protamine. This was done by giving successive doses and checking the clotting time for several minutes after each dose. This dog survived for 24 hours and appeared to be in very good condition, not considering the effects of the recent coronary occlusion.
SUMMARY

The results of the present set of experiments may be summarized as follows:

1. Changes have been determined in the systemic arterial and retrograde coronary arterial plasma of dogs following coronary occlusion. These changes are:
   a. an increase in lactic acid
   b. an increase in amino acids
   c. an increase in glucose
   d. an increase in lactic dehydrogenase
   e. an increase in glutamic oxaloacetic transaminase
   f. a decrease in bicarbonate
   g. a decrease followed by an increase in magnesium
   h. an increase in calcium
   i. a slight increase in pyruvate
   j. no change in chloride

2. Certain differences between retrograde and arterial plasma composition have been found and their significance as evidence of passage of blood through exchange channels has been discussed.

3. Certain simultaneous relationships between the effects of coronary occlusions and those of exogenous catecholamines strongly suggest that the alterations in plasma constituents which occur within the first three hours following coronary
occlusion are mediated by catecholamines.

4. The effects of certain procedures which reduce catecholamine availability or decrease their effects on the changes in plasma composition following coronary occlusion have been investigated. These procedures and their effects are:
   a. adrenalectomy; does not abolish the alterations in plasma composition
   b. pre-treatment with reserpine; does not abolish the alterations in plasma composition
   c. pre-treatment with dibenzyline; appears to reduce the alterations in plasma composition

   The relationship of each of these effects to the general response has been discussed.

5. The oxygen saturation of retrograde blood has been determined. The results demonstrate a tendency for the retrograde blood to be lower in percent oxygen saturation than the arterial.

6. Measurements of retrograde blood flow rate and peripheral-coronary arterial pressure following coronary occlusion have been presented. Both of these parameters vary widely in different dogs.
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VITA

Albert Jerome Bocage was born in New Orleans, Louisiana on April 8, 1930. He attended the public school system of Orleans Parish, graduating from McDonogh #35 High School in June, 1947. After a period of uninteresting occupations and serving in the Army from 1951 to 1953, he enrolled at Xavier University of New Orleans and graduated as a Bachelor of Science in Chemistry in June, 1957. He was subsequently employed as a research technologist in the Department of Physiology in Louisiana State University School of Medicine. After resigning the position, he enrolled in September, 1960 as a graduate student in the same department as a candidate for the degree of Master of Science in Physiology, which he obtained in August, 1962. He then continued as a candidate for the degree of Doctor of Philosophy.
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Title of Thesis: Chemical Changes in Blood and Heart Tissue in Experimental Coronary Occlusion

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