Influence of Increased Glucose and Insulin Administration on Certain Chemical Constituents of Normal and Coronary Occluded Heart of the Dog

Blanche Tigerman

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INFLUENCE OF INCREASED GLUCOSE AND INSULIN ADMINISTRATION
ON CERTAIN CHEMICAL CONSTITUENTS OF NORMAL AND
CORONARY OCCLUDED HEART OF THE DOG

by
Blanche Tigerman

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
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Master of Science

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LIFE

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CHAPTER I

INTRODUCTION AND THE STATEMENT OF THE PROBLEM

The mammalian heart requires a steady supply of energy yielding substances for the maintenance of normal function. For a number of years, attention has been directed at carbohydrate compounds such as glucose, glycogen, and lactic acid in connection with energy sources. More recently, high energy compounds have been examined also since they have been shown to play a central role in the energy metabolism of living cells. Briefly, the chemical energy released during the degradation of carbohydrate, fat, and protein is localized in the high energy phosphate molecule which represents the form, in which chemical energy can be utilized in physical processes such as muscular contraction.

A fundamental physiological problem in energy utilization is the elucidation of mechanisms which control the supply of energy yielding substances in the mammalian heart. In intact animals, a conventional approach to this study involves the determination of the level of certain compounds before and after
producing an environmental change. If a change in concentration results, it can be concluded that the experimental variation may be an important factor in the control of the energy supply. The purpose of the present investigation was to study the influence of glucose and insulin addition on the concentration of certain important constituents of the intact dog heart, such as glycogen, lactic acid, high energy phosphate, and potassium. The significance of this study lies in the better understanding of the control of energy yielding materials in the normal dog heart.

The administration of glucose has been shown previously to cause a glycogen elevation in cardiac as well as in skeletal muscle. In the diabetic state, where the tissues are exposed to high blood sugar, cardiac glycogen is elevated, but strangely, glycogen in skeletal muscle is decreased.

The influence of glucose administration on lactic acid and high energy phosphate has not been studied extensively in heart tissue. In the explanation of the effect of hyperglycemia on the energy yielding substances, it is possible that the participation of the cation, potassium, will be an important consideration. It is known, for example, that potassium uptake is increased when glucose uptake is high.

An important aspect in the present investigation will
be the study of the influence of glucose and insulin infusion on all of these compounds in the same tissue sample. The correlation of separate findings by several different laboratories may lead to error due to varying conditions.

A final object in this study will be to determine if glucose infusion may exert a protective influence on experimental coronary occlusion. Since it has been shown that degradative changes in these constituents occur in the occlusion, it was of interest to us to determine if these changes could be reversed by glucose administration to the occluded heart.
CHAPTER II

REVIEW OF LITERATURE

A. The Relationship between Glucose Utilization and High Energy Phosphate Metabolism.

A thorough search of literature reveals that very little attention has been directed to the study of phosphate metabolism in the intact myocardium of animals given glucose or insulin. However, related work is available on the perfused dog heart by Follack and co-workers (1933-34). On the basis of these experiments, it appears that phosphate metabolism in the heart may be indeed influenced by carbohydrate metabolism. In experiments where the heart was perfused with blood alone, the value for blood sugar gradually decreased (20 mg.\% in 5 hours) with a simultaneous decrease in serum inorganic phosphate. On the addition of 1.0 gm. of glucose every half hour to the perfusing blood (1.0 liter), there would be a sudden increase in the strength of the heart beat, and upon chemical analysis, these hearts all contained elevated phosphocreatine (PC; 16-30 mg.\% from a normal range of 8-12 mg.\%). Insulin (2 units every half hour) did not seem to have any additional effect.

During the course of perfusion experiments on the
isolated hind limb of the dog Polleak and co-workers (1933-34) observed that there is a fall of the serum phosphate level when glucose is added, similar to that observed in the intact animal. When insulin (2 units every half hour) and 750 mg. of inorganic phosphate were added with the glucose to the perfusing blood, no change was found in the inorganic phosphate (IP) content of the muscle. In both types of experiments there was an increase in the content of PC of the skeletal muscle.

In normal, depancreatized, and adrenalectomized dogs insulin caused a significant fall in serum IP (Soskin and co-workers, 1941). Glucose and epinephrine were effective in the normal and adrenalectomized dogs but not in the depancreatized. It is apparent that the fall in IP occurs only in the presence of the pancreas or of administered insulin. Thus, the fall of IP in blood appears to be related to insulin. The fall of IP following an injection of epinephrine in normal dogs is a result either of a rise in the blood sugar level or to a reflex secretion of insulin consequent to hyperglycemia.

It has been shown that the serum phosphate changes following intravenous injections of glucose (2.0 gm./kg. of body weight per hour; length of infusion 4-12 hours) seem to have a capacity time factor (Polleak et. al., 1933-34). That is, some time after the injection period, there is a return to the
normal from depressed level. The site of deposition of this
phosphate is presumably in the muscle.
B. The Relationship between Glucose Utilization and Glycogen
Content.

The glycogen content of the mammalian heart varies
somewhat as to the species of animal used. The distribution of
glycogen in the mammalian heart has been studied by several
(Cruickshank and Shrivastava, 1930; MacPherson, Essex, and Mann,
1951). The concentration is generally highest in the auricles,
next in the septum and lowest in the ventricles. This may be
related to the difference in work performed by each of these
portions of the heart.

Excised and perfused hearts show greater variations
in their glycogen content. MacPherson and co-workers (1951)
found that more glycogen disappears in the first hour of perfusion
than at any succeeding time. This may be attributed to the
relative hypoxia. Glycogen disappears faster when the hearts are
perfused with solution free from glucose. Sprague (1953) devised
a modified perfusion fluid by the addition of washed RBC to
Ringer's solution, which increased the vigor and rate of perfused
hearts and prevented the initial decrease in the glycogen content
of the heart muscle. Addition of glucose to perfusion fluid
containing RBC appeared to retard the depletion of glycogen from
the perfused heart.

Evans (1934) found in normal rats that feeding glucose does not increase cardiac glycogen. However, if insulin is injected at the same time that glucose is fed, cardiac glycogen is increased. A few years later (1941) the same author reported that intravenous glucose caused a definite increase of cardiac glycogen in the rat. Insulin did not seem to cause any further increase in cardiac glycogen. This led the author to a conclusion that insulin is not necessary for the deposition of cardiac glycogen.

Stimulation of the splanchnic nerve of normal animals leads to a decrease in glycogen of skeletal muscle. In starved animals there is either a slight decrease or no change in glycogen in muscles. In both types of animals an increase of hexose phosphate is observed (Yakolev, 1937).

Starvation affects the heart glycogen of normal animals; it raises the cardiac glycogen to a high value similar to that of a diabetic dog. The high level of cardiac glycogen in the case of diabetes is not increased anymore. Liver glycogen is lost in starvation in both the normal and diabetic animal and an increase of ketone bodies results (Fisher and Lackey, 1925). These findings are in agreement with those of Macleod and Prendergast (1921), Visscher and Mulder (1930), Cruickshank (1936).
and Lackey (1947). Similar results are reported by Long and Evans (1932-33), who, experimenting on rats, observed a progressive increase in the glycogen content of the heart during fasting, while that of skeletal muscle diminished.

Experimental diabetes has been an important tool for carbohydrate research for a long time. The first experimental production of a syndrome resembling severe diabetes mellitus in the human was reported by von Mering and Minkowski in 1889, who produced this condition by removing the pancreas. Minkowski found that the percentage of liver glycogen of the diabetic dog fell 0.5% or less regardless of the amount of dextrose ingested.

Fisher and Lackey in 1925 conducted a complete study of glycogen in heart and liver in normal and diabetic dogs. They showed by their results that the disappearance of glycogen from the liver is more rapid in case of the diabetic than in the normal animal. Cardiac glycogen is increased in diabetes, but when insulin is given, the level of glycogen approaches that of normal dogs.

Gruickshank and Shrivastava (1930) established both for the normal and diabetic heart that sugar utilization is increased by the addition of insulin and also by maintaining the blood sugar concentration at a high level. They also demonstrated that the diabetic heart perfused with diabetic blood will not utilize
blood sugar and that this loss of power is restored by perfusion with normal blood or by the addition of insulin. Whether or not under experimental conditions in a normal heart there is a synthesis or breakdown of glycogen apparently depends upon insulin dosage and blood sugar concentration. It was found by these investigators, that the diabetic heart, to which insulin is administered in physiological doses, maintains glycogen synthesis and brings the glycogen and muscle sugar content toward the normal.

As a brief summary comment of the literature concerned with glycogen, it can be stated that intravenous injection of glucose raises cardiac glycogen, while oral ingestion has no effect. Insulin given together with intravenous glucose will not increase the glycogen level further.

C. The Relationship between Glucose Utilization and Cation Changes.

The known facts concerning the physiology of potassium from the point of view of any well developed theory of potassium behavior are still very limited. Many attempts have been made to clarify the behavior of potassium (K) and sodium (Na), but many discrepancies are found in the literature to this effect. The majority of these studies is chiefly concerned with the K changes in the serum and not in the tissue.
Many investigators have shown that muscular activity leads to a loss of K in exchange for Na. The increase of K in the blood has been found in isolated perfused muscles of dogs (Wood et al., 1939), in rats (Kendall and co-workers, 1937), and in man after exercise (Keys, 1937).

The fall of sugar resulting from insulin is accompanied by a simultaneous fall of K and inorganic phosphate (Harrop et al., 1924; Kerr, 1928; Keys, 1938). The injection of glucose into the blood of cats and rats lowers K as if sugar were being deposited in tissues in combination with K (Flock et al., 1958). Green and co-workers (1951-52) concluded from their results on human patients that decreases in serum K and inorganic phosphate are physiological phenomena which occur regularly when glucose is assimilated by muscle cells under the influence of insulin; the glucose is administered either by vein or mouth to normal individuals and to non-diabetic patients.

Thus, from the rather limited number of studies on electrolytes present in the literature one can expect a fall in serum K when glucose is given. In other instances, it will also fall with simultaneous fall of blood sugar.

D. Metabolism of Coronary Occluded Heart.

After coronary occlusion was produced in dogs, Himwich et al. (1934) found a depletion of glycogen and accumulation of
lactic acid in the ischemic area. These findings agree with those of Tennant et al. (1936) who in addition studied the chemical changes after removal of the ligatures. The lactic acid content fell below the normal level, accounted for by being washed out by the reestablishment of blood flow, and not by resynthesis of the lactic acid to glycogen, because the latter values remained low after the restoration of the circulation. On the contrary, Hastings et al. (1939) reported the content of glycogen and potassium to be unaffected in the occluded area. However, it should be pointed out, that the coronary artery in this case was ligated only for 45 minutes, then released, and the heart removed several hours later.

The arrest of the uptake of the lactic acid is accompanied by the destruction of DPN (diphosphopyridinenucleotide, coenzyme I), the coenzyme of lactic dehydrogenase (Govier, 1945).

In the terminal stages of asphyxia of the isolated dog heart there is a production of lactic acid. The survival of the heart is prolonged by increased glucose concentration in blood, but is not affected by the presence of lactate. Glycogen depletion is prevented by moderate concentration of glucose and lactate, or by high concentration of lactate alone (Bogue et al., 1938). More recent studies of severe long-lasting anoxia indicates that in order to survive this condition the minimal
level of cardiac glycogen has to be approximately 200 mg%.
(Cordier, D., and Dessaux, G., 1951).

In chronic myocardial occlusion there is a definite
decrease in K level of heart or blood with an increase in Na
concentration (Alexander et. al., 1950; Iseri et. al., 1952).
CHAPTER III

PROCEDURES AND METHODS

A. Experimental Procedure.

In order to determine the amount of glucose to be given, the pertinent literature was reviewed. In order to prevent death due to hypoglycemia in hepatectomized animals, it was found by Mann and co-workers (1927) that this condition could be eliminated on administration of 250 mg./kg. of glucose. This figure has been confirmed by other investigators in similar experiments (Yater et. al., 1933), and also was in agreement with Soskin's work (1937), in which the utilization of carbohydrate by the peripheral tissues was estimated by the method of chemical balance.

In our experiments we intended to produce hyperglycemia within a short time, thus it was necessary to overload the animal with glucose. An infusion of glucose at a rate of 1.6 gm./kg./hr. for a period of 5 hours was decided upon. This amount of glucose agrees with that of Pollack (1933-34) who added that quantity of glucose to perfusing blood in his study of serum phosphate changes.

Mongrel dogs, weighing ten to twenty kilograms, were
anesthetized with 28 mg./kg. nembutal intraperitoneally. A cannula was inserted into the trachea and artificial respiration applied. The femoral artery was exposed and cannulated for recording blood pressure. Twenty per cent solution of glucose was given into the femoral vein at a rate of 8 ml./kg./hr. for 5 hours. This hypertonic glucose solution was decided upon in order to minimize the volume of fluid given. Blood samples were taken at one hour intervals. Essentially the same procedure was followed in the animals infused with insulin as well as glucose, except that 125 units of insulin were mixed with each 100 ml. of glucose. The total amount of insulin given in such a manner was 625 units. At the end of the infusion period, the thoracic cavity was opened, the pericardium slit and the heart was rapidly removed and dropped into a freezing mixture of dry ice and ether. The time required for the removal of the heart did not exceed five seconds. A short period for removal is necessary due to the unstable nature of high energy phosphates.

In one group of animals, experimental coronary occlusion was produced. This usually produced a darkened area of about the size of 1/2 inch in diameter. Of twenty animals in this group, three animals died of ventricular fibrillation due to coronary occlusion, and two others were not used, because the blood pressure fell below the normal level during the experiment.
After the pericardium was opened, a branch of the left anterior descending coronary artery was ligated. At the time of ligation intravenous physiological saline infusion was begun at the rate of 8 ml./kg./hr. and the animal was maintained in this condition for four hours. Blood pressure was recorded from the cannulated femoral artery with aid of a mercury manometer in order to evaluate the condition of the animal continuously. The same procedure was applied to another group of animals with coronary occlusion with the exception infusing glucose instead of saline.

B. Preparation of the Heart Samples for Analysis.

Samples of the right and left ventricles were chipped off from the frozen heart. In those, in which myocardial occlusion was produced, portions of the ischemic and unaffected sections of the left ventricle were taken. All samples were kept in beakers with the freezing mixture after they were cleared of papillary muscle, and excessive blood and fat.

Approximately 2.5-3.0 gram samples were used for phosphate analyses. The tissue was placed into a pre-cooled mortar and was macerated with a small amount of 5% trichloracetic acid (TCA). The mixture was filtered through phosphate-free filter paper and collected in a graduated glass-stoppered cylinder. The mortar and filter paper were washed with several consecutive small quantities of TCA until the volume of the filtrate was
45-50 ml. The extract was neutralized to phenolphthalein with 30% KOH, brought to a volume of 50 ml. and stored in the refrigerator. In the experiments in which the occlusion was produced, a 5 ml. aliquot was taken for the lactic acid determination before neutralization.

Due to the unstable nature of the high energy phosphates, the preparation of the samples was completed within 30 minutes and all operations were carried out in a deep freeze at 0°C.

Two 1.0 gram samples of each ventricle were weighed out for the determination of glycogen as well as for the analysis of sodium and potassium. The tissue for glycogen was placed into warm 5.0 ml. of 30% KOH; the samples set aside for the electrolyte analysis were placed into a digesting tube and were tightly stoppered.

C. Chemical Determinations.

1. Phosphate Compounds

Inorganic phosphate (IP), the phosphorus of phosphocreatine (PC), the phosphorus of adenosine polyphosphate (APP) were the phosphate fractions determined. Inorganic phosphate, determined by the method of Fiske and Subbarow (1929), is precipitated as the calcium salt. Two 1.0 ml. of the extract is added 0.2 ml. of 10% CaCl₂. After 5 minutes standing at room
temperature, the slowly formed precipitate is centrifuged for 15 minutes, decanted, and 0.5 ml. of 3.5% CaCl₂ solution is added. This mixture is re-centrifuged and the supernatant decanted. The washed precipitate is then dissolved with 5 drops of 10% HCl and the solution is brought to 3.0 ml. volume with distilled water.

The following reagents are then added in order: 0.4 ml. of 10 N H₂SO₄, 0.8 ml. of 3.5% of ammonium molybdate, 0.4 ml. of the reducing mixture (2,4-aminonapthol-sulfonic acid). The contents are diluted to a final volume of 10 ml. with distilled water, and read against a blank in the spectrophotometer at a wave length of 660 millimicrons after allowing the color to develop for 10 minutes. The inorganic phosphate estimation is based on the color reaction of Fiske and Subbarow (1925). The basis of the color reaction is the production of a blue color with the simultaneous reduction of molybdic acid in the presence of acid.

The phosphorus of PC is estimated, applying the method of Fiske and Subbarow (1929), by subtracting the value obtained for the IP from a value obtained in a following manner: 1.0 ml. of the extract is diluted to 5.0 ml., 0.4 ml. of 10 N H₂SO₄ and 0.8 ml. of 3.5% ammonium molybdate are added respectively. Standing at room temperature for 30 minutes will allow for the hydrolysis of PC. At the end of this period, 0.4 ml. of the
Reducing agent is added, volume is adjusted to 10 ml, and after the full color development is read again as before.

The method of Lohmann (1928) is utilized for the determination of APP. This fraction is also obtained by difference, this time subtracting the value of the 30 minutes hydrolysis from the value obtained in the 8 minutes acid hydrolysis at 100°C. The latter is carried out as follows: 1.0 ml. of 2 N HCl is added to 1.0 ml. of the extract, and this mixture is hydrolyzed in a boiling water bath for exactly 8 minutes. After cooling in tap water, 1.0 ml. of distilled water, 0.4 ml. of 10 N H₂SO₄, 0.8 ml. of 2.5% ammonium molybdate, and 0.4 ml. of the reducing agent are added with shaking. After bringing the volume of the mixture to 10.0 ml. with distilled water, and allowing the color to fully develop, the contents are read again in the spectrophotometer as before.

When recovery experiments were conducted on these methods, the results agreed with those reported in literature (Folkesk, 1933-34; Wollenberger, 1947), the recovery of IF being 98-102%, the recovery of RC 83-88%, and that of APP being 85-88%.

2. Glycogen

Glycogen is hydrolyzed by the method of Good et. al. (1935) and the glucose is determined according to Nelson method (1944).
Two and a half hour digesting in a boiling water bath dissolves the tissue contained in 50% KOH. After filtration through glass wool, the contents of the tube are diluted to 20.0 ml., and 5.0 ml. aliquots are taken for analysis. To each aliquot 5.0 ml. of 95% alcohol is added, mixed well, and brought slowly to boiling in a water bath to precipitate out the glycogen. The tubes are cooled in the refrigerator for 30 minutes, and then centrifuged for 10 minutes. The supernatant is decanted, and 5.0 ml. of 2 N HCl is added to the precipitated glycogen. Two and a half hour hydrolysis in a boiling water bath follows for the conversion of glycogen to glucose.

The hydrolysate is neutralized to phenolphthalein with 1% NaOH and diluted to a final volume of 10 ml. with distilled water. One ml. aliquots are taken for the glucose determination according to Nelson's method. One ml. of CuSO₄ reagent is mixed with each aliquot, and the tubes are placed in a boiling water bath for exactly 30 minutes. After cooling in tap water, addition of 1.0 ml. of arseno-molybdate reagent follows. The tubes are shaken well until the evolution of CO₂ ceases, and then the contents are brought to a volume of 25.0 ml.

The resulting stable bluish-green color is read in the spectrophotometer against a blank at a wave length of 520 milli-microns.
3. **Blood Glucose**

One ml. of blood is mixed well with 8.0 ml. N/12 H₂SO₄ and 1.0 ml. of 10% sodium tungstate is added, and vigorously shaken to precipitate the proteins present. Filtration follows and 1.0 ml. aliquot is taken for the glucose determination, as described above.

4. **Lactic Acid**

A modified method of Miller and Muntz (1938) is used for the determination of lactic acid. To a clean, dry test tube containing 0.2 ml. of the filtrate, 7.0 ml. of H₂SO₄ reagent is slowly added with constant shaking. The H₂SO₄ reagent is composed of 1.0 liter of concentrated H₂SO₄, 117 ml. of distilled water, and 5.6 ml. of aqueous 5% CuSO₄. After heating 6 minutes in a boiling water bath, the mixture is rapidly cooled below 20°C in a bath containing running tap water. Two drops of the color reagent 1.5% p-hydroxydiphenyl solution is added, tubes are well shaken, and kept for 30 minutes in the dark in a water bath at 30°C. Then, they are heated in a boiling water bath for 90 seconds, cooled in tap water, and read against a water blank in the spectrophotometer at wave length of 565 millimicrons.

5. **Electrolytes**

Five ml. of concentrated HNO₃ is used for digestion of the tissue samples. After about 2 hours digestion, the volume is
decreased to approximately 1.0 ml. The digest is brought to 25.0 ml. volume, 10.0 ml. aliquots are taken and diluted in volumetric flasks to 50.0 ml. To each flask, before adjusting the final volume, 1.0 ml. of 2% lithium lactate is added, serving as the internal standard (Overman and Davis, 1947). The diluted samples are then ready for analysis of sodium and potassium in the Perkin Elmer flame photometer.
CHAPTER IV

RESULTS

A total of 46 dogs were studied in the evaluation of the influence of glucose infusion on the chemical constituents of the normal and coronary occluded dog heart. Since the amount of data and the number of cross-comparison is likely to confuse the reader, the following procedure will be used in describing the results.

The phosphate results will be described for the normal and compared with the data in (1) glucose infused animals, (2) glucose-insulin infused animals, and (3) the unaffected area of saline infused open-chest animals. Then, the results in the unaffected area of the saline infused open-chest animals will be compared with (1) the unaffected area of the glucose infused open-chest animals, and (2) the ischemic area of saline infused open-chest animals. Finally, the affected area of the saline infused open-chest animals will be compared with the ischemic area of the glucose infused open-chest animals. This order of presentation will be repeated for the glycogen and lactic acid results, and will be followed by the same procedure for the electrolytes.
A. The Effect of Infusing Glucose with and without Insulin on the Phosphate Fractions of Normal and Coronary Ocluded Dog Heart.

The concentration of the phosphate compounds in normal dog hearts is shown in Table I. The values for the left ventricle are essentially the same as those reported by Wollamerger (1947). It is interesting to note that the concentration values for the right and left ventricle are about the same.

In those animals given glucose infusion there are some interesting changes from the normals as may be seen in Table II. For the right ventricle, there is a decrease in IP, an increase in PC, and no change in APP. The left ventricle shows a similar trend although only the increase in PC approaches borderline statistical significance.

In the presence of insulin as well as glucose, essentially the same changes in the phosphate levels are seen. In this series, reported in Table III, the PC level is definitely higher than in the previous series with glucose alone. The IP in the left ventricle is lower than the control values, while APP remains unchanged. Pollack and co-workers (1954) found similar results in dog hearts perfused with blood and glucose; insulin and phosphate did not seem to have any additional effect. The PC content was higher in the hearts perfused with glucose
than with blood alone.

In the series of animals studied with coronary occlusion, saline infusion was given in one group of animals so that the volume of fluid administered would be comparable to those given glucose. These results are found in Table IV. No other results are found in the literature in this connection, except the ones from this laboratory reported previously (Mulder et al., 1950), and those agree with the present results. The analysis of the right ventricle in this type of experiment was omitted because the content of the phosphate fractions remains unchanged under these conditions (Mulder et al., 1950). The comparison between the ischemic area and the unaffected area of the left ventricle shows a definite decrease in PC and APP, and no difference in IP. If one compares the results of the unaffected left ventricle with the ones of the left ventricle of normal dogs (Table I), one notices immediately an increase in IP, while no change is apparent in PC and APP. This increase in IP is, however, statistically not significant.

Concentrations of phosphate compounds in coronary occluded hearts of dogs given glucose infusion are tabulated in Table V. When compared with the same phosphate compounds of the hearts of dogs receiving saline infusions (Table IV) the only statistically important value is found in the non-affected left
ventricle in PC content, which is increased. The IP tends to decrease and APP content is slightly elevated. This compares with infusion into normal hearts. The comparison of the ischemic area and non-affected area of the left ventricle shows a marked loss of PC and APP, and an increase in IP. Apparently glucose infusion does not protect against coronary occlusion.

It can be stated from the summary Table VI, that in the glucose and glucose-insulin infused normal animals, all PC values increased while APP and IP tended to decrease in its concentration.

Summary of the phosphate compounds of the coronary occluded hearts is found in Table VII. When the occluded and non-occluded areas of the same left ventricle in each group, i.e. saline and glucose infused, are statistically analyzed, both PC and APP contents of the ischemic area in both groups are markedly low, while IP concentration tends to rise. A comparison made between the saline and glucose infused hearts shows only one marked change, namely, the increase in concentration of PC in the non-affected left ventricle of the glucose infused animals. There is a definite trend for IP to decrease and APP to rise in the non-affected left ventricle.
B. The Effect of Infusing Glucose with and without Insulin on the Glycogen and Lactic Acid of Normal and Coronary Ocluded Dog Hearts.

Table VIII represents the values for normal glycogen and lactic acid. These values compare with those reported in the literature (Vischer and Mulder, 1930; Cruickshank and Shrivastava 1930). It is interesting to note that the content of glycogen is slightly higher in the right ventricle than in the left ventricle.

When glucose infusion is given, blood glucose is about three-four fold of its normal value and cardiac glycogen is increased (Table IX). These results are in agreement with those reported in the literature in diabetic hearts (Fisher and Lackey, 1925; Cruickshank and Shrivastava, 1930; Evans - in rat, 1941).

Addition of insulin to the glucose infusion will not raise the cardiac glycogen any more; in fact, there seems to be a tendency for the level of glycogen to fall to a normal one (Table X). Similar results were observed by Evans (1941) who made these same observations in rats.

Concentrations of glycogen and lactic acid in coronary occluded hearts of dogs given saline infusion are given in Table XI, those given glucose infusion are found in Table XII. In both, the ischemic area of the left ventricle is deficient of
glycogen, and shows an accumulation of lactic acid, especially
in Table XII, where glucose is infused.

If one correlates the results of the glucose infused
coronary hearts with those infused with saline, there is hardly
any change in glycogen concentration, while there is surpris-
ingly an increase of lactic acid in glucose infused experimental
animals in both sections of the left ventricle.

After coronary occlusion was produced in dogs Himwich
et. al. (1934) found that glycogen decreases and lactic acid
is produced in the occluded area, while Hastings et. al. (1939)
oberved that the glycogen content of the occluded area was
unaffected. None of these animals received glucose infusion.

As a summarizing statement it can be said that glucose
infusion will raise cardiac glycogen, while insulin infused with
glucose will not raise its concentration further, on the contrary,
may lower it slightly (Table XIII); the glucose infusion in the
coronary occluded hearts does not raise the glycogen, but only
appears to increase the lactic acid concentration (Table XIV).

C. The Effect of Infusing Glucose with and without Insulin on
the Electrolyte of Normal and Coronary Occluded Dog Hearts.

Control values for sodium and potassium are found in
Table XV. These values are comparable with those found in the
literature (Boyer and Poindexter, 1940; Wood and Moe, 1942).
Sodium remains more or less unchanged, while potassium is significantly increased in both ventricles, when glucose and glucose-insulin mixture are infused (Tables XVI, XVII).

Upon comparison with normal concentration of sodium and potassium (Table XV) with those obtained in coronary occlusion with saline infusion, a rise of both electrolytes is observed in the non-occluded area of the left ventricle, while there is no change in the ischemic area of the left ventricle (Table XVIII). Similar results are found in experimental coronary occlusion with glucose infusion (Table XIX). Glucose infusion does not change the concentrations of either electrolyte as compared with the saline infusion. No significant change results in sodium when each of the occluded area is compared with the non-affected area, but there is a loss of potassium in the affected area. These results are contradicted by those of Hastings et al. (1939), who found potassium to be unaffected in the ischemic area. It should be pointed out, however, that the experimental conditions were quite different. Hastings occluded the artery for only 45 minutes, then released it, and removed the heart several hours later; also, no saline or glucose infusion was given.

As a summarizing statement it can be said, that glucose and glucose-insulin infusion will not alter the content of sodium but will raise the potassium concentration in a normal heart.
(Table XX). In the coronary occluded hearts receiving saline and others receiving glucose infusion, both sodium and potassium concentrations are increased, the latter to a lesser extent in the ischemic area.
### TABLE I. CONCENTRATIONS (mg%) OF PHOSPHATE COMPOUNDS IN THE HEARTS OF NORMAL DOGS.

<table>
<thead>
<tr>
<th>DOG NO.</th>
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<th>RIGHT VENTRICLE</th>
</tr>
</thead>
<tbody>
<tr>
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<td>PC</td>
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<tr>
<td>1</td>
<td>34.3</td>
<td>19.3</td>
</tr>
<tr>
<td>2</td>
<td>24.3</td>
<td>8.3</td>
</tr>
<tr>
<td>3</td>
<td>25.4</td>
<td>14.7</td>
</tr>
<tr>
<td>6</td>
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<td>9.4</td>
</tr>
<tr>
<td>14</td>
<td>23.2</td>
<td>8.5</td>
</tr>
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<td>17</td>
<td>24.1</td>
<td>9.2</td>
</tr>
<tr>
<td>20</td>
<td>26.7</td>
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<td>11.6</td>
</tr>
<tr>
<td>29</td>
<td>25.0</td>
<td>9.3</td>
</tr>
</tbody>
</table>

| Mean   | 26.7 | 10.7 | 30.0 | 20.1 | 12.5 | 30.2 |
| 3. E. of Mean | ±1.2 | ±1.1 | ±1.3 | ±1.0 | ±1.2 | ±1.4 |

**IP**: Inorganic Phosphate.

**PC**: Phosphocreatine.

**APP**: Adenosine Polyphosphate.

**mg%**: Milligrams of Phosphorus per 100 grams of Tissue.
<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>AMOUNT INFUSED (ml)</th>
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<th>RIGHT VENTRICLE</th>
</tr>
</thead>
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<td></td>
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<td>480</td>
<td>24.9</td>
<td>18.0</td>
</tr>
<tr>
<td>12</td>
<td>520</td>
<td>25.4</td>
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<td>23.8</td>
<td>16.4</td>
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<td>18</td>
<td>960</td>
<td>15.6</td>
<td>23.6</td>
</tr>
<tr>
<td>19</td>
<td>720</td>
<td>21.4</td>
<td>12.6</td>
</tr>
</tbody>
</table>

Mean: . . . . . . . . . . . . . . . . . . . . . . . . . . 23.3 14.7 30.8 20.6 20.9 29.3
S. E. of Mean: . . . . . . ±1.2 ±1.4 ±1.1 ±1.0 ±1.1 ±1.4
p Value: . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . <.01 <.01 .6

p Value refers to the probability that the samples were drawn from the same population as the control hearts and was evaluated by Fisher's t test.
### TABLE III. CONCENTRATIONS (mg%) OF PHOSPHATE COMPOUNDS IN THE HEARTS OF DOGS RECEIVING A CONTINUOUS GLUCOSE-INSULIN (625 UNITS) FOR A PERIOD OF FIVE HOURS.

<table>
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<th>DOG NO.</th>
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<th>RIGHT VENTRICLE</th>
</tr>
</thead>
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<td></td>
<td>IP</td>
<td>PC</td>
</tr>
<tr>
<td>21</td>
<td>520</td>
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<td>24.4</td>
</tr>
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<td>440</td>
<td>22.6</td>
<td>16.0</td>
</tr>
<tr>
<td>23</td>
<td>480</td>
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</tr>
<tr>
<td>25</td>
<td>440</td>
<td>23.6</td>
<td>22.7</td>
</tr>
<tr>
<td>26</td>
<td>480</td>
<td>27.0</td>
<td>22.4</td>
</tr>
<tr>
<td>27</td>
<td>640</td>
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</tr>
<tr>
<td>30</td>
<td>640</td>
<td>17.5</td>
<td>22.9</td>
</tr>
<tr>
<td>31</td>
<td>720</td>
<td>13.8</td>
<td>10.3</td>
</tr>
<tr>
<td>32</td>
<td>380</td>
<td>30.5</td>
<td>12.2</td>
</tr>
</tbody>
</table>

<p>| Mean     | 20.9 | 19.4 | 25.6 | 22.9 | 23.7 | 50.5 |
| S. E. of Mean | ±1.6 | ±1.8 | ±2.4 | ±2.0 | ±2.9 | ±1.9 |
| p Value   | &lt;.02 | &lt;.01 | .3   | .1   | &lt;.01 | .2   |</p>
<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>AMOUNT INFUSED ML.</th>
<th>LEFT VENTRICLE IP</th>
<th>PC</th>
<th>APP</th>
<th>LEFT OCCLUDED VENTRICLE IP</th>
<th>PC</th>
<th>APP</th>
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<tr>
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<td>32.0</td>
<td>0.4</td>
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<td>28.6</td>
<td>20.5</td>
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<td>38.6</td>
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<td>15.9</td>
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<td>560</td>
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<td>10.0</td>
<td>56.9</td>
<td>36.2</td>
<td>6.6</td>
<td>16.7</td>
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</table>

**Mean:** 32.9 11.1 29.9 55.7 4.2 13.9  
**S. E. of Mean:** ±3.7 ±2.2 ±3.3 ±1.9 ±.75 ±2.8  
**p Value:** .1 .5 .6 .6 .01 .01
<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>AMOUNT INFUSED ML.</th>
<th>LEFT VENTRICLE IP</th>
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<th>LEFT OCCLUDED VENTRICLE IP</th>
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<td>44.5</td>
<td>26.4</td>
<td>10.9</td>
<td>26.7</td>
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</table>

Mean: 24.9 23.9 37.4 37.5 5.8 14.6
S. E. of Mean: ±1.8 ±2.6 ±3.4 ±2.4 ±1.0 ±3.7
p Value: .1 .01 .2 .6 .6 .8
TABLE VI. SUMMARY OF THE MEANS OF PHOSPHATE CONCENTRATIONS (mg%) IN GLUCOSE INFUSED AND GLUCOSE-INSULIN INFUSED AND CONTROL ANIMALS.

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<td>GLUCOSE-INSULIN INFUSED</td>
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<td>GLUCOSE INFUSED</td>
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TABLE VII. SUMMARY OF THE MEANS OF PHOSPHATE CONCENTRATIONS (mg%) OF CORONARY OCCLUDED HEARTS RECEIVING GLUCOSE AND THOSE RECEIVING SALINE.

<table>
<thead>
<tr>
<th></th>
<th>LEFT VENTRICLE</th>
<th>LEFT OCCLUDED VENTRICLE</th>
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<td>GLUCOSE INFUSED</td>
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TABLE VIIIb. SUMMARY OF THE MEANS OF PHOSPHATE CONCENTRATIONS (mg%) OF CORONARY OCCLUDED HEARTS RECEIVING GLUCOSE AND THOSE RECEIVING SALINE. (Statistical comparison is made on the occluded area versus the non-affected area.)

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<th>GLUCOSE INFUSION</th>
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<td>24.9</td>
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<tr>
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</tr>
<tr>
<td>APP</td>
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<td>37.4</td>
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### TABLE VIII. CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN THE HEARTS OF NORMAL DOGS.

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<td>GLYCOGEN</td>
<td>LACTIC ACID</td>
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<td>604</td>
<td>12.7</td>
<td>658</td>
<td>12.5</td>
</tr>
<tr>
<td>17</td>
<td>670</td>
<td>10.0</td>
<td>761</td>
<td>14.2</td>
</tr>
<tr>
<td>20</td>
<td>425</td>
<td>17.2</td>
<td>499</td>
<td>15.3</td>
</tr>
<tr>
<td>24</td>
<td>809</td>
<td>17.0</td>
<td>740</td>
<td>15.8</td>
</tr>
<tr>
<td>29</td>
<td>560</td>
<td>17.8</td>
<td>923</td>
<td>27.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean.</th>
<th></th>
<th>Mean.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>619</td>
<td>13.8</td>
<td>720</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>±40</td>
<td>±0.6</td>
<td>±34</td>
<td>±1.2</td>
</tr>
</tbody>
</table>
### TABLE IX. CONCENTRATIONS (mg%) OF GLYCOGEN AND MEAN BLOOD GLUCOSE IN THE HEARTS OF DOGS RECEIVING A CONTINUOUS GLUCOSE INFUSION FOR A PERIOD OF FIVE HOURS.

<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>LEFT VENTRICLE</th>
<th>RIGHT VENTRICLE</th>
<th>MEAN BLOOD GLUCOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1128</td>
<td>1353</td>
<td>240</td>
</tr>
<tr>
<td>5</td>
<td>1060</td>
<td>1205</td>
<td>400</td>
</tr>
<tr>
<td>7</td>
<td>954</td>
<td>1161</td>
<td>410</td>
</tr>
<tr>
<td>8</td>
<td>935</td>
<td>1504</td>
<td>303</td>
</tr>
<tr>
<td>10</td>
<td>1164</td>
<td>1217</td>
<td>465</td>
</tr>
<tr>
<td>11</td>
<td>937</td>
<td>1209</td>
<td>493</td>
</tr>
<tr>
<td>12</td>
<td>1117</td>
<td>1444</td>
<td>423</td>
</tr>
<tr>
<td>13</td>
<td>810</td>
<td>1167</td>
<td>343</td>
</tr>
<tr>
<td>15</td>
<td>1012</td>
<td>900</td>
<td>462</td>
</tr>
<tr>
<td>16</td>
<td>1505</td>
<td>963</td>
<td>303</td>
</tr>
<tr>
<td>18</td>
<td>1107</td>
<td>1276</td>
<td>473</td>
</tr>
<tr>
<td>19</td>
<td>836</td>
<td>963</td>
<td>595</td>
</tr>
</tbody>
</table>

Mean: . . . . . . . 1034  1204  391
S. E. of Mean: ± 42  ± 52  ± 23
p Value: . . . . . . < .01  < .01
### TABLE X. CONCENTRATIONS (mg%) OF GLYCOGEN AND MEAN BLOOD GLUCOSE IN THE HEARTS OF DOGS RECEIVING A CONTINUOUS GLUCOSE—INSULIN (625 UNITS) FOR A PERIOD OF FIVE HOURS.

<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>LEFT VENTRICLE</th>
<th>RIGHT VENTRICLE</th>
<th>MEAN BLOOD GLUCOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>938</td>
<td>1094</td>
<td>395</td>
</tr>
<tr>
<td>22</td>
<td>713</td>
<td>1305</td>
<td>343</td>
</tr>
<tr>
<td>23</td>
<td>630</td>
<td>1106</td>
<td>338</td>
</tr>
<tr>
<td>25</td>
<td>1060</td>
<td>1065</td>
<td>401</td>
</tr>
<tr>
<td>26</td>
<td>900</td>
<td>1093</td>
<td>452</td>
</tr>
<tr>
<td>27</td>
<td>930</td>
<td>1328</td>
<td>233</td>
</tr>
<tr>
<td>28</td>
<td>742</td>
<td>938</td>
<td>460</td>
</tr>
<tr>
<td>30</td>
<td>751</td>
<td>1133</td>
<td>228</td>
</tr>
<tr>
<td>31</td>
<td>903</td>
<td>1189</td>
<td>214</td>
</tr>
<tr>
<td>32</td>
<td>957</td>
<td>915</td>
<td>452</td>
</tr>
</tbody>
</table>

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>874</td>
<td>1121</td>
<td>341</td>
</tr>
<tr>
<td><strong>S. E. of Mean</strong></td>
<td>±46</td>
<td>±41</td>
<td>±35</td>
</tr>
<tr>
<td><strong>p Value</strong></td>
<td>.2</td>
<td>.01</td>
<td></td>
</tr>
</tbody>
</table>
TABLE XI.  CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN  
CORONARY OCCLUDED HEARTS OF DOGS GIVEN SALINE  
INFUSION.

<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>LEFT VENTRICLE</th>
<th>LEFT OCCLUDED VENTRICLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GLYCOGEN</td>
<td>LACTIC ACID</td>
</tr>
<tr>
<td>33</td>
<td>755</td>
<td>19.5</td>
</tr>
<tr>
<td>54</td>
<td>712</td>
<td>16.2</td>
</tr>
<tr>
<td>35</td>
<td>768</td>
<td>22.0</td>
</tr>
<tr>
<td>38</td>
<td>825</td>
<td>15.3</td>
</tr>
<tr>
<td>41</td>
<td>787</td>
<td>25.0</td>
</tr>
<tr>
<td>44</td>
<td>758</td>
<td>21.7</td>
</tr>
<tr>
<td>46</td>
<td>706</td>
<td>20.4</td>
</tr>
</tbody>
</table>

Mean: 784  20.0  532  30.8  
S. E. of Mean: 119  1.2  1.7  1.8  
p Value: .1  .05  .01  .01
### TABLE XII. CONCENTRATIONS (mg%) OF GLYCOGEN AND LACTIC ACID IN CORONARY OCCLUDED HEARTS OF DOGS GIVEN GLUCOSE INFUSION.

<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>LEFT VENTRICLE</th>
<th>LEFT OCCLUDED VENTRICLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GLYCOGEN</td>
<td>LACTIC ACID</td>
</tr>
<tr>
<td>36</td>
<td>562</td>
<td>24.3</td>
</tr>
<tr>
<td>37</td>
<td>878</td>
<td>51.3</td>
</tr>
<tr>
<td>39</td>
<td>927</td>
<td>47.5</td>
</tr>
<tr>
<td>40</td>
<td>956</td>
<td>28.7</td>
</tr>
<tr>
<td>42</td>
<td>950</td>
<td>29.2</td>
</tr>
<tr>
<td>43</td>
<td>579</td>
<td>42.2</td>
</tr>
<tr>
<td>45</td>
<td>964</td>
<td>39.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean.</th>
<th>S. E. of Mean.</th>
<th>p Value.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>618</td>
<td>±64</td>
<td>.6</td>
</tr>
<tr>
<td>Lactic ACID</td>
<td>34.6</td>
<td>±5.2</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Glycogen</td>
<td>598</td>
<td>±46</td>
<td>.2</td>
</tr>
<tr>
<td>Lactic ACID</td>
<td>65.2</td>
<td>±13.2</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>
TABLE XIII. SUMMARY OF THE MEANS OF GLYCOGEN (mg%) IN GLUCOSE INFUSED AND GLUCOSE-INSULIN INFUSED AND CONTROL ANIMALS.

<table>
<thead>
<tr>
<th></th>
<th>LEFT VENTRICLE</th>
<th>RIGHT VENTRICLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL MEAN</td>
<td>619</td>
<td>720</td>
</tr>
<tr>
<td>GLUCOSE INFUSED</td>
<td>1034</td>
<td>1204</td>
</tr>
<tr>
<td>GLUCOSE-INSULIN</td>
<td>874</td>
<td>1121</td>
</tr>
</tbody>
</table>

TABLE XIVa. SUMMARY OF THE MEANS OF GLYCOGEN AND LACTIC ACID CONCENTRATIONS (mg%) OF CORONARY OCCLUDED HEARTS RECEIVING GLUCOSE AND THOSE RECEIVING SALINE.

<table>
<thead>
<tr>
<th></th>
<th>LEFT VENTRICLE</th>
<th>LEFT OCCLUDED VENTRICLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLYCOGEN</td>
<td>LACTIC ACID</td>
<td>GLYCOGEN</td>
</tr>
<tr>
<td>SALINE INFUSED</td>
<td>784</td>
<td>20.0</td>
</tr>
<tr>
<td>GLUCOSE INFUSED</td>
<td>618</td>
<td>34.6</td>
</tr>
<tr>
<td></td>
<td>.6</td>
<td>.01</td>
</tr>
</tbody>
</table>
TABLE XIVb. SUMMARY OF THE MEANS OF GLYCOGEN AND LACTIC ACID CONCENTRATIONS (mg%) OF CORONARY OCCLUDED HEARTS RECEIVING GLUCOSE AND THOSE RECEIVING SALINE. (Statistical comparison is made on the occluded area versus the non-occluded area.)

<table>
<thead>
<tr>
<th></th>
<th>SALINE INFUSION</th>
<th>GLUCOSE INFUSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEFT VENTRICLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLYCOGEN</td>
<td>794</td>
<td>816</td>
</tr>
<tr>
<td>LACTIC ACID</td>
<td>20.0</td>
<td>34.6</td>
</tr>
<tr>
<td>LEFT OCCLUDED VENTRICLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLYCOGEN</td>
<td>532 .01</td>
<td>398 .01</td>
</tr>
<tr>
<td>LACTIC ACID</td>
<td>30.6 .01</td>
<td>65.2 .01</td>
</tr>
</tbody>
</table>
### TABLE XV. CONCENTRATIONS (meq./kg.) OF SODIUM AND POTASSIUM IN THE HEARTS OF NORMAL DOGS.

<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>LEFT VENTRICLE</th>
<th>RIGHT VENTRICLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SODIUM</td>
<td>POTASSIUM</td>
</tr>
<tr>
<td>1</td>
<td>29.1</td>
<td>63.7</td>
</tr>
<tr>
<td>2</td>
<td>37.7</td>
<td>69.5</td>
</tr>
<tr>
<td>3</td>
<td>36.3</td>
<td>65.3</td>
</tr>
<tr>
<td>6</td>
<td>33.1</td>
<td>62.8</td>
</tr>
<tr>
<td>9</td>
<td>29.4</td>
<td>65.5</td>
</tr>
<tr>
<td>14</td>
<td>32.8</td>
<td>67.1</td>
</tr>
<tr>
<td>17</td>
<td>33.7</td>
<td>61.7</td>
</tr>
<tr>
<td>20</td>
<td>33.1</td>
<td>62.2</td>
</tr>
<tr>
<td>24</td>
<td>32.6</td>
<td>66.7</td>
</tr>
<tr>
<td>29</td>
<td>33.1</td>
<td>59.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean.</th>
<th>S. E. of Mean.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SODIUM</td>
<td>33.6</td>
<td>±.9</td>
</tr>
<tr>
<td>POTASSIUM</td>
<td>64.9</td>
<td>±1.8</td>
</tr>
<tr>
<td>SODIUM</td>
<td>37.7</td>
<td>±1.7</td>
</tr>
<tr>
<td>POTASSIUM</td>
<td>65.4</td>
<td>±1.7</td>
</tr>
<tr>
<td>DOG NO.</td>
<td>LEFT VENTRICLE</td>
<td>RIGHT VENTRICLE</td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td>SODIUM</td>
<td>POTASSIUM</td>
</tr>
<tr>
<td>4</td>
<td>35.4</td>
<td>31.3</td>
</tr>
<tr>
<td>5</td>
<td>37.2</td>
<td>31.3</td>
</tr>
<tr>
<td>7</td>
<td>33.6</td>
<td>35.3</td>
</tr>
<tr>
<td>8</td>
<td>32.2</td>
<td>32.6</td>
</tr>
<tr>
<td>10</td>
<td>38.0</td>
<td>35.8</td>
</tr>
<tr>
<td>11</td>
<td>33.5</td>
<td>33.6</td>
</tr>
<tr>
<td>12</td>
<td>38.0</td>
<td>32.8</td>
</tr>
<tr>
<td>13</td>
<td>31.8</td>
<td>37.0</td>
</tr>
<tr>
<td>15</td>
<td>23.7</td>
<td>22.1</td>
</tr>
<tr>
<td>16</td>
<td>43.0</td>
<td>40.3</td>
</tr>
<tr>
<td>18</td>
<td>54.6</td>
<td>53.3</td>
</tr>
<tr>
<td>19</td>
<td>28.3</td>
<td>27.6</td>
</tr>
</tbody>
</table>

Mean. . . . . . . . 35.7  32.0  35.2  78.6
S. E. of Mean. . . ±2.2  ±2.7  ±2.1  ±1.5
p Value . . . . . . . <.01  <.01  <.01  <.01
TABLE XVII. CONCENTRATIONS (meq./kg.) OF SODIUM AND POTASSIUM IN THE HEARTS OF DOGS RECEIVING A CONTINUOUS GLUCOSE-INSULIN (625 UNITS) FOR A PERIOD OF FIVE HOURS.

<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>LEFT VENTRICLE</th>
<th>RIGHT VENTRICLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SODIUM</td>
<td>POTASSIUM</td>
</tr>
<tr>
<td>21</td>
<td>34.2</td>
<td>75.6</td>
</tr>
<tr>
<td>22</td>
<td>30.3</td>
<td>76.2</td>
</tr>
<tr>
<td>23</td>
<td>32.1</td>
<td>80.1</td>
</tr>
<tr>
<td>24</td>
<td>29.8</td>
<td>75.4</td>
</tr>
<tr>
<td>25</td>
<td>31.5</td>
<td>81.8</td>
</tr>
<tr>
<td>26</td>
<td>29.1</td>
<td>84.0</td>
</tr>
<tr>
<td>27</td>
<td>35.6</td>
<td>75.4</td>
</tr>
<tr>
<td>28</td>
<td>15.1</td>
<td>67.1</td>
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<tr>
<td>29</td>
<td>32.4</td>
<td>76.0</td>
</tr>
<tr>
<td>30</td>
<td>29.0</td>
<td>68.2</td>
</tr>
</tbody>
</table>

Mean: 29.9 76.0 35.9 77.5
S. E. of Mean: ±1.7 ±1.8 ±1.9 ±2.1
p Value: .1 <.01 <.01
TABLE XVIII. CONCENTRATIONS (meq./kg.) OF SODIUM AND POTASSIUM IN CORONARY OCCLUDED HEARTS OF DOGS GIVEN SALINE INFUSION.

<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>LEFT VENTRICLE</th>
<th>LEFT OCCLUDED VENTRICLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SODIUM</td>
<td>POTASSIUM</td>
</tr>
<tr>
<td>33</td>
<td>49.8</td>
<td>72.1</td>
</tr>
<tr>
<td>34</td>
<td>47.2</td>
<td>105.6</td>
</tr>
<tr>
<td>35</td>
<td>45.8</td>
<td>92.9</td>
</tr>
<tr>
<td>38</td>
<td>36.0</td>
<td>91.8</td>
</tr>
<tr>
<td>41</td>
<td>53.4</td>
<td>87.5</td>
</tr>
<tr>
<td>44</td>
<td>44.1</td>
<td>93.4</td>
</tr>
<tr>
<td>46</td>
<td>45.7</td>
<td>83.1</td>
</tr>
</tbody>
</table>

Mean: .... 46.0 89.6 50.5 67.0
S. E. of Mean: ±2.0 ±3.2 ±1.2 ±4.7
<table>
<thead>
<tr>
<th>DOG NO.</th>
<th>LEFT VENTRICLE</th>
<th>LEFT OCCLUDED VENTRICLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SODIUM</td>
<td>POTASSIUM</td>
</tr>
<tr>
<td>36</td>
<td>48.9</td>
<td>80.2</td>
</tr>
<tr>
<td>37</td>
<td>25.8</td>
<td>97.3</td>
</tr>
<tr>
<td>39</td>
<td>31.5</td>
<td>107.4</td>
</tr>
<tr>
<td>40</td>
<td>32.1</td>
<td>90.7</td>
</tr>
<tr>
<td>42</td>
<td>49.6</td>
<td>75.0</td>
</tr>
<tr>
<td>43</td>
<td>49.5</td>
<td>85.1</td>
</tr>
<tr>
<td>45</td>
<td>48.0</td>
<td>87.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>S. E. of Mean</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SODIUM</td>
<td>45.5</td>
<td>±2.7</td>
<td>.1</td>
</tr>
<tr>
<td>POTASSIUM</td>
<td>72.6</td>
<td>±4.6</td>
<td>.4</td>
</tr>
</tbody>
</table>
TABLE XX. SUMMARY OF THE MEANS OF SODIUM AND POTASSIUM CONCENTRATIONS (meq./kg.) IN GLUCOSE INFUSED AND GLUCOSE-INSULIN INFUSED AND CONTROL ANIMALS.

<table>
<thead>
<tr>
<th></th>
<th>LEFT VENTRICLE</th>
<th></th>
<th>RIGHT VENTRICLE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SODIUM</td>
<td>POTASSIUM</td>
<td>SODIUM</td>
<td>POTASSIUM</td>
</tr>
<tr>
<td>CONTROL</td>
<td>35.6</td>
<td>64.9</td>
<td>37.7</td>
<td>65.4</td>
</tr>
<tr>
<td>INFUSED</td>
<td>35.7</td>
<td>82.0</td>
<td>35.2</td>
<td>78.6</td>
</tr>
<tr>
<td>GLUCOSE</td>
<td>34</td>
<td>.01</td>
<td>4</td>
<td>.01</td>
</tr>
<tr>
<td>INSULIN</td>
<td>29.9</td>
<td>78.0</td>
<td>35.9</td>
<td>77.5</td>
</tr>
<tr>
<td>INFUSED</td>
<td>1</td>
<td>.01</td>
<td>5</td>
<td>.01</td>
</tr>
</tbody>
</table>

TABLE XXIa. SUMMARY OF THE MEANS OF SODIUM AND POTASSIUM CONCENTRATIONS (meq./kg.) OF CORONARY OCCLUDED HEARTS RECEIVING GLUCOSE AND THOSE RECEIVING SALINE.

<table>
<thead>
<tr>
<th></th>
<th>LEFT VENTRICLE</th>
<th>LEFT OCCLUDED VENTRICLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SODIUM</td>
<td>POTASSIUM</td>
</tr>
<tr>
<td>INFUSED</td>
<td>46.0</td>
<td>89.6</td>
</tr>
<tr>
<td>GLUCOSE</td>
<td>39.9</td>
<td>87.6</td>
</tr>
<tr>
<td>INFUSED</td>
<td>1</td>
<td>.7</td>
</tr>
</tbody>
</table>
# TABLE IXIB. SUMMARY OF THE MEANS OF SODIUM AND POTASSIUM CONCENTRATIONS (meq./kg.) OF CORONARY OCCLUDED HEARTS RECEIVING GLUCOSE AND THOSE RECEIVING SALINE. (Statistical comparison is made on the occluded area versus the non-affected area.)

<table>
<thead>
<tr>
<th></th>
<th>SALINE INFUSED</th>
<th>GLUCOSE INFUSED</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LEFT VENTRICLE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SODIUM</td>
<td>46.0</td>
<td>39.9</td>
</tr>
<tr>
<td>POTASSIUM</td>
<td>59.6</td>
<td>67.6</td>
</tr>
<tr>
<td><strong>LEFT OCCLUDED VENTRICLE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SODIUM</td>
<td>50.5</td>
<td>45.5</td>
</tr>
<tr>
<td></td>
<td>*1</td>
<td>*2</td>
</tr>
<tr>
<td>POTASSIUM</td>
<td>67.0</td>
<td>72.6</td>
</tr>
<tr>
<td></td>
<td>*0.01</td>
<td>*0.03</td>
</tr>
</tbody>
</table>
CHAPTER V

DISCUSSION

It is well established that the energy-rich phosphate compounds play an important role in the physiology of muscle (Wollenberger, 1947). It was our hope in this investigation to obtain some insight as to the mechanisms which operate in maintaining the level of these as well as other energy-yielding substances.

In the present study the concentration of energy-yielding substances was investigated in two separate conditions. First, an environment, which would enhance glucose utilization, was accomplished by the addition of glucose with or without insulin to normal anesthetized animals. Second, a low oxygen environment was achieved in a portion of the myocardium by experimental coronary ligation. It is believed that a state of hypoxia exists rather than anoxia, due to the presence of anastomotic channels. It was of interest to us further to determine if glucose infusion would aid this situation of aerobic processes in the metabolism of the occluded dog heart.
Influence of Increased Glucose Utilization on Chemical Constituents of the Normal Heart.

A. Phosphate Compounds

According to Soskin (1937), the presence of increased amounts of glucose or insulin will increase the rate of glucose utilization. In the present study, the addition of glucose alone resulted in a higher level of phosphocreatine (PC), and in a decline of inorganic phosphate (IP) (Fig.1). This indicates that an increased phosphorylation took place as a result of the increased glucose utilization by the cell. Using radioactive phosphorus ($P^{32}$), Kaplan and Greenberg (1944) found that the combination of glucose and insulin produces the greatest increases in the total acid-soluble $P^{32}$ and the $P^{32}$ of the labile phosphate of ATP in the liver. The increase in $P^{32}$ in the insulin-glucose-administered animals, according to the above authors, may represent an enhanced oxidation of the administered glucose. The work of Sacks (1943) with radioactive phosphorus shows that insulin plus glucose increases the uptake of $P^{32}$ in the skeletal muscle of the intact animal, which this author interprets as increase in rate of turnover of the phosphate in adenosine triphosphate (ATP). This interpretation is not warranted on the basis of this evidence since the incorporation of $P^{32}$ may be indirectly related to a greater penetration of $P^{32}$. 
**Fig. 1**

**Summary of Results**

N - normal  
G - glucose infused  
G-I - glucose plus insulin infused  
S - saline infused  
C - coronary occlusion  
C-G - coronary occlusion plus glucose
The constant level of adenosine polyphosphate (APP) with an increase in phosphocreatine (PC) in our results appears to be consistent with the concept that ascribes a storage role of PC. An increase of APP may be possible only by raising the level of adenine nucleotide. On the other hand, free creatine appears to be available under normal conditions. These findings support essentially the current opinion regarding the energy metabolism of tissues, viz. APP is a common step in synthesis and utilization, PC is a storage form, and IP is a necessary precursor of high energy phosphate.

When insulin is added to the glucose infusing mixture, the same changes occur in the phosphate fractions, but to a more extensive degree than when glucose alone is infused. That is, the level of PC is increased further and that of IP declines further. Insulin, therefore, appears to augment glucose oxidations and the associated formation of high energy phosphate. In the experiments with glucose alone, the hyperglycemia undoubtedly influences the pancreas to elaborate insulin, so that the hormone is present in these experiments as well.

B. Glycogen

The results of this study confirm essentially the results reported in the literature (Cruickshank, 1936; Cruickshank and Startup, 1935; Evans, 1941; Fisher and Lackey, 1925). Intravenous glucose infusion into normal animals increases the
level of cardiac glycogen, addition of insulin has no further effect on its increase. In fact, it tends to bring the glycogen level closer to the normal. However, this may be attributed to the fact that less glucose is present in the blood when insulin is added, due to increased utilization by liver and skeletal muscle.

Despite the lower glycogen content, insulin may act to promote the cellular utilization of glucose. This is supported by the observation that the FC level is higher in the presence of insulin than in hearts infused with glucose alone, at least in the left ventricle. Under the condition of low glycogen content it is likely that increased phosphorylation may mean that there is increased utilization of glucose. This would be indicated better by further study with radioactive glucose (Chernick and Chaikoff, 1950).

C. Electrolytes

In the present study, sodium (Na) changes did not take place. Since a significant portion of the Na exists as extracellular Na, and very little of it as intracellular, it is conceivable that our present method of analysis is not sensitive enough to detect any appreciable change in the intracellular Na concentration.

Increased uptake of potassium (K) with increased
glucose utilization is well documented (Szent-Györgyi, 1947). In glucose infusion, it has been found (Pollack, 1933-34) that the decrease in serum inorganic phosphate is accompanied by a fall of serum K. This decrease of serum K, as postulated by the above author, may cause an increase of the K in the tissues, but no experimental data are offered as evidence. The rise in tissue K content, in our present study, may thus be a result of active K incorporation due to the increased glucose utilization.

Glucose-insulin infusion raises the potassium level to a lesser extent than glucose infusion alone, and lowers the sodium content below the normal. It is interesting to note that potassium and glycogen changes appear to vary in the same direction. Hastings (1959) has reported the necessity of K for glycogen synthesis in liver slices. The decrease in sodium is not statistically significant but considering the difficulty in detecting intracellular sodium changes, it may be worth noting. The increased amount of chemical energy may be related to a greater active extrusion of sodium from the heart cell.

The Influence of Saline Infusion on the Chemical Constituents of the Hearts of Open-Chest Animals.

One of the objectives of the present study was to determine the influence of glucose infusion on chemical constituents of the coronary occluded heart. If complete infarction
would exist, after a period of four hours one would expect no
phosphocreatine (PC) to be present and very low concentration of
adenosine polyphosphate (APP), if any. In these experiments,
it was decided to keep the chests partially open in order to
observe the extent of cyanosis produced by the ligation. In
order to have an adequate control for the glucose infused
coronary occluded animals, an identical amount of saline was
infused into another group of animals. In each of these groups,
portions of the non-affected left ventricle were taken to serve
as internal controls for the occlusion. Since the chemical
results of the non-affected portion of the ventricle of saline
infused animals were not completely comparable to those found in
intact closed chest animals, a brief discussion of these results
is in order.

In the saline infused animals the increase in sodium
(Na) may possibly be a result of sodium chloride per se. Even
if intracellular sodium may not increase, the extracellular
space may simply increase in the tissue. Potassium in the un-
affected area, however, is higher than in normals. This
increase is not apparently accountable by loss of potassium (K)
from the occluded area, since K here is at normal levels.

The most plausible interpretation of the electrolyte
results may require a consideration of a further factor, viz.
a relative dehydration of the cells. In the open-chest experiments, a considerable amount of water may be lost by the exposed tissues, even though the surface of the heart itself was kept moist with saline and the chest roughly approximated with hemostats. This would tend to cause cell dehydration, and as a result the intracellular Na and K concentrations may be expected to increase. Saline infusion is apt not to reduce this condition, although by increasing the extracellular fluid space it may tend to cause a reduction in tissue K and an increase in Na. Apparently this latter change is insufficient to offset the change in cellular dehydration.

The Influence of Glucose Infusion on the Non-Occluded Area of the Heart of Open-Chest Dogs.

When glucose is infused into the open-chest dogs, a rise in PC and APP with simultaneous decrease in IP is observed in the unaffected area of the left ventricle when compared with the saline infused open-chest dogs. The rise in APP was not seen in the normal intact chest experiments and is somewhat difficult to explain. Perhaps, the concentration of adenylic acid or adenosine diphosphate is greater under these circumstances.

In the unaffected area of the left ventricle, the glycogen content is in the normal range as in the case of saline
infusion, while lactic acid concentration is greater. It is reasonable to believe that glycogen content should have been greater. The fact that the glycogen level remained virtually the same while lactic acid increased, suggests that there is greater glycogenolysis in the glucose infused hearts. Glucose infusion should, if anything, improve the dehydration since glucose penetrates the cell membrane readily. It is difficult to see why glycogen breakdown should be greater than during the saline infusion.

The sodium values appeared to be less but potassium was just as high. The Na decrease may be related to a relative decrease in the extracellular fluid space and perhaps to a greater extrusion from the cell. The failure for K to increase as compared to the saline infused animals is ascribed to the same reason given for the failure of glycogen level to increase.

The Influence of Coronary Occlusion on the Hearts of Saline Infused Open-Chest Animals.

In the affected area of the left ventricle in saline infused animals, when compared with the unaffected section of the same ventricle, both PC and APP are significantly decreased, while IP is increased. This is undoubtedly due mainly to failure in synthesis of high energy phosphate.
Since there is an inadequate supply of oxygen in the ischemic area, glycogen breakdown results as well as an increase in the accumulation of lactic acid. Similar results were obtained by Hinwich (1934).

The potassium decrease, then, can be correlated with the diminution of glycogen. Since sodium tends to enter the cell when potassium leaves, our finding of an increase is not unusual.

The Influence of Glucose Infusion on the Coronary Occluded Area of the Hearts of Open-Chest Animals.

Glucose infusion did not alter the concentration of the phosphate fractions of the ischemic area; the results were the same as those of the saline infused experiments.

There is a further degradation of glycogen with a very marked rise in lactic acid concentration when glucose is infused. This increase in glycogenolysis was observed in the non-affected regions also and is difficult to resolve. It may be possible that a portion of the glycogen may be relatively unchanging whereas a second portion is labile. In both saline and glucose infusions the glycogen recorded may be the stable fraction. In the presence of glucose, more labile glycogen may be formed but it may be broken down immediately, so that a finite increase in content is not recordable. This may,
therefore, account for the increased lactic acid content.

The slight elevation of K and the fall of Na in the glucose infused ischemic area is consistent with the finding in the non-affected areas and the same explanations given there apply here also.

Glucose infusion which has a noticeable influence on the readily available energy supply in the normal heart does not appear to influence the concentrations of energy-rich phosphate compounds or glycogen in experimental coronary occlusion.

Comment on the Interpretation of Simultaneous Chemical Findings.

The determination of chemical constituents on the same tissue is, of course, essential for an attempt of correlating changes. The primacy of any single event is difficult to determine unless time course studies are done. Since this was not carried out in the present study, the initial change preceding all others cannot be accurately assessed. As a best approximation, it appears likely that glucose transport causes plasma potassium to enter which in turn may be instrumental in promoting net glycogen synthesis. The simultaneous increase in glucose oxidation may account for the increased phosphorylation.

In coronary occlusion glucose transport is undoubtedly decreased causing small changes in glycogen and electrolytes.
The relative lack of oxygen will inhibit phosphorylation as well.
CHAPTER VI

SUMMARY

1. (a) Glucose or glucose plus insulin was administered intravenously for five hours to normal dogs. Saline or glucose was infused for four hours to another group of animals in which coronary occlusion was produced by occluding a branch of the left anterior descending coronary artery.

(b) Analysis of the frozen tissue for inorganic phosphate, phosphocreatine, adenosine polyphosphate, glycogen, lactic acid, sodium, and potassium were carried out. In normal animals, glucose infusion raises the level of phosphocreatine, has no effect on adenosine polyphosphate, and tends to decrease inorganic phosphate in the dog heart.

When insulin is added to the glucose infusion, a still greater increase in phosphocreatine results, adenosine polyphosphate remains unchanged, and a further decrease in inorganic phosphate is observed.

(c) Glucose infusion markedly elevates cardiac glycogen, while glucose-insulin infusion raises glycogen to
a lesser extent. The latter effect may be due to blood sugar rising less in the presence of insulin.

(d) Tissue sodium, under the influence of glucose, remains the same; potassium is increased in its concentration. Insulin addition to the glucose infusion mixture lowers the sodium content and raises potassium level to a lesser degree than glucose infusion alone.

2. (a) In the coronary occlusion experiments, saline infusion has no effect on the phosphocreatine content and adenosine polyphosphate, while inorganic phosphate is increased in the non-affected portion of the left ventricle.

(b) The content of cardiac glycogen is slightly raised.

(c) Both sodium and potassium increase during saline infusion. These effects are considered to be related to a non-specific factor, viz. cellular dehydration in the open-chest dogs.

3. (a) Glucose infusion affects the area of these hearts, which is not occluded, in a similar manner as it affects the normal heart, i.e. the phosphocreatine content is raised, while the inorganic phosphate is lowered. In addition, the content of adenosine polyphosphate is raised.
(b) Glycogen remains the same as in the saline infusion, while lactic acid is raised under the influence of glucose.

(c) No change in potassium is observed upon comparison with saline infusion; sodium tends to decrease.

4. (a) In the occluded area of the saline infusion experiments, phosphocreatine and adenosine polyphosphate are markedly decreased, inorganic phosphate content is elevated.

(b) A decrease in glycogen content is observed, lactic acid is slightly elevated upon comparison with the non-affected section of the ventricle.

(c) The potassium concentration was less than the value in the non-affected area of the same ventricle; sodium was raised.

5. (a) No change is found in all the phosphate fractions determined under glucose infusion in the ischemic section.

(b) Glycogen breakdown and lactic acid formation is greater than when saline is administered.

(c) Potassium is slightly higher and sodium lower in glucose infusion.

6. As a summarizing statement, it can be concluded that glucose and glucose plus insulin infusion alter certain
chemical constituents of the dog heart in a favorable direction, i. e. synthesis.

Glucose infusions have little significant effect on the occluded area although the infusion affects the non-occluded area of these hearts in a manner similar to that in normal hearts.
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II. SECONDARY SOURCES


APPROVAL SHEET

The thesis submitted by Blanche Tigerman has been read and approved by three members of the Department of Physiology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

January 3, 1955

Date

Signature of Advisor